ACNFP

Advisory Committee on Novel Foods and Processes

Annual Report 2000

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Advisory Committee on Novel Foods and Processes – Annual Report 2000

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FOOD STANDARDS AGENCY

The Advisory Committee on Novel Foods and Processes (ACNFP) is an independent body of experts whose remit is:

'to advise the central authorities responsible, in England, Scotland, Wales and Northern Ireland respectively on any matters relating to novel foods and novel food processes including food irradiation, having regard where appropriate to the views of relevant expert bodies.'

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Foreword

2000 was another productive year for the Advisory Committee on Novel Foods and Processes. In April the FSA was formally established and I have been delighted to brief the Board on Novel Food issues and to welcome the Board member with special responsibility for Novel Foods, who has attended one of our meetings and who keeps in regular contact with the Committee.

In recent years we have endeavoured to make our work more accessible and transparent and to this end we now make as much information as possible available to the public before we meet. In addition to the agenda, minutes and Secretariat papers, novel food marketing applications (excluding the minimum of commercial confidential data) are now placed on the web for public comment. We do hope that there is a positive response to this initiative and assure all respondees that the Committee will consider their comments carefully.

In addition to our mainstream tasks in relation to the applications for marketing novel foods and new processing methods, we continue to monitor current research projects, for example the Safety of Novel Foods research programme, and identify possible emergent techniques that could be used to supplement the safety assessment process. We also continue to advise the FSA on a wide range of other important generic issues outlined in this report. Dialogue with other food advisory committees, and cross committee membership of individuals continues to be essential, as we all deal with issues related to novel foods in a consistent, robust and rigorous manner.

I would like to take this opportunity to thank my fellow Committee Members for their support, hard work and expert advice throughout the past year. All Members have given freely of their time and played a full part in the safety assessment process and consideration of related issues; in addition to scientific advice the ethical and consumer representatives have made a major contribution to all our discussions and decision making.

Finally, I wish to record my thanks and appreciation to the hard working members of the Secretariat. Their help and support has been highly professional and invaluable to the effective operation of the Committee.

Professor J. M Bainbridge O.B.E.

Introduction

This is the twelfth annual report of the work of the Advisory Committee on Novel Foods and Processes (ACNFP). The report begins with an overview of the EC Regulation on Novel Foods and Novel Food Ingredients (258/97) which came into force on 15 May 1997.

The ACNFP received a number of applications in 2000, details of which are at Sections 2, and 3 of this report. Section 2 contains reports of full applications initially received by the UK Competent Authority; Section 3 contains details of reports on applications made to other Member States, who provided the initial opinion. Those topics discussed during 2000 that were continuations of previous work are indicated as such.

The Committee also discussed a number of general issues during the year, including Cholesterol lowering Spreads, further information on these issues can be found at Section 4.

As part of the Committee's commitment to increasing the transparency of its work, it took the unprecedented step of releasing to the public all non-confidential information contained in applications to the UK Competent Authority. Public comments on the applications are invited, and taken into account when the Committee makes its assessment. In 2000 the Committee published both the dossier on Trehalose and the dossier on Echium Oil and these can be found on the ACNFP pages of the Food Standards Agency Website.

A cumulative index of topics considered in previous annual reports can be found at Section 11. Copies of previous annual reports can be obtained from the Secretary to the Committee (see Section 7). The Committee's last three annual reports, as well as other information can be found on its website at *www.foodstandards.gov.uk/committees/acnfp/summary.htm*

1 The EC Novel Foods Regulation (258/97)

1.1 The Regulation

On 15 May 1997, Regulation (EC) 258/97 of the European Parliament and of the Council, concerning Novel Foods and Novel Food Ingredients[1] came into effect introducing a statutory pre-market approval system for novel foods throughout the European Union. This Regulation is directly applicable and legally binding in all Member States, and in the UK replaced the voluntary scheme for the assessment of novel foods which had been in operation for more than 10 years. Under the EC Novel Foods Regulation, companies wishing to market a novel food in the EU are required to submit an application to the Competent Authority in the Member State where they first intend to market their product. In the UK the Competent Authority is provided by the Food Standards Agency.

The Regulation 258/97 defines a novel food as a food which has no significant history of human consumption within the Community prior to May 1997, and which falls under one of the following categories:

(a) foods and food ingredients containing or consisting of GMOs within the meaning of Directive 90/220/EEC;

b) foods and food ingredients produced from, but not containing GMOs;

c) foods and food ingredients with a new or intentionally modified primary molecular structure;

d) foods and food ingredients consisting of, or isolated from micro-organisms, fungi or algae;

e) foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagation or breeding practices and having a history of safe food use;

f) foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances.

Where there is any doubt whether a food is novel or not, the EC Standing Committee for Foodstuffs will decide.

1.2 Implementing the Regulation: Full and substantial equivalence applications.

The implementation of the EC Novel Foods Regulation has brought changes to the ACNFP and the way that it operates. Although most applications are discussed at a formal meeting, due to the statutory time limits imposed by the Regulation (i.e. 90 days for initial opinion and 60 days for assessment of opinions expressed by other Member States), it has been necessary for consideration of some applications to be completed between meetings. Members may discuss applications and other issues that arise between meetings, although the Committee's conclusions are published in the usual way.

The safety assessment of novel foods follows a comparative approach set out by the EC guidelines[2] (details of which are available from the Stationary Office or the ACNFP Secretariat, see page 22). Wherever possible, the novel food is compared with an existing counterpart, which it may replace in the diet. Differences between a novel food and its counterpart are identified and undergo a detailed examination in order to establish whether the novel food is as safe as its conventional counterpart.

For a full safety assessment, companies are required to submit an application to the appropriate Competent Authority in the Member State where they first intend to market the product. A copy of the application must also be sent to the European Commission. Once a Competent Authority has accepted an application, it has 90 days in which to complete an initial safety assessment and forward it to the Commission. The Commission must then copy the assessment to other Member States for their comments, which have to be made within 60 days. If the initial assessment is favourable and no objections are raised by other Member States, then the food product can be marketed. If objections are raised, or if the initial Member State considers that an additional assessment is required, the application will be referred to the EC Standing Committee for Foodstuffs for final agreement, consulting the EC Scientific Committee for Food as necessary.

Under article 3 (4) of the Novel Foods and Novel Food Ingredients Regulation (258/97)[1] a simplified procedure exists whereby a company can notify the Commission that they intend to place a product in categories b, d or e (see page 1) on the market. With such a notification the supporting evidence can be based upon the opinion of a Member State or on generally available and recognised scientific evidence. The evidence must

show that the novel food or food ingredient is substantially equivalent to an existing food or food ingredient as regards to composition, nutritional value, metabolism, intended use and the level of undesirable substances it contains.

In December 1997 the ACNFP looked at the issue of notifications, which could be considered under this procedure. They concluded that in their opinion, for food ingredients derived from GM crops, only those which contained no DNA or protein would be suitable for considering under such a procedure. The Standing Committee for Foodstuffs has now agreed with this approach.

All other ingredients derived from GM crops where novel DNA or novel protein may be present (as a result of less intensive processing compared with refined foods) would not be able to be assessed under this procedure and would require an application for full safety assessment to be made.

1.3 Applications to the ACNFP under the previous voluntary scheme for the safety assessment of novel foods.

A number of products were considered by the ACNFP under the voluntary safety assessment scheme, which operated before the EC Novel Foods Regulation (258/97) came into force in May 1997. A list of these is contained in the 1996 ACNFP annual report[11]. Those products that were known to have been marketed before May 1997 have been indicated on this list with an asterisk. Copies can be obtained from the ACNFP Secretariat (see page 22).

Under the Novel Foods Regulation, even if a product had been cleared previously for food use the product's safety would require reassessment, if it had not been marketed within the EU before May 1997. Products marketed prior to the introduction of the Novel Foods Regulation do not require reassessment by the ACNFP or another EU Competent Authority but remain, in the UK, subject to the provisions of the UK Food Safety Act (1990)[3].

2 Full applications submitted to the UK Competent Authority

2.1 Trehalose

The ACNFP was asked to consider an application for approval of Trehalose, a sugar produced by a novel enzymatic process from food grade starch. The process uses four enzymes that have not been evaluated by the UK Food Advisory Committee (FAC); however, it was noted that a subsequent purification step in the production process would remove all proteinaceous material from the final product. This application was first considered by the ACNFP at it's July meeting, and the additional data sought were considered by Members by correspondence.

This was the first application to the ACNFP for an initial opinion on a novel food to be handled under the new openness procedures agreed in December 1999. The application dossier, minus sections deemed to be commercially confidential, was placed on the ACNFP website for public comment on 25 May, the first day of the 90-day evaluation period: *(www.foodstandards.gov.uk/committees/acnfp/trehalose.htm)*.

The Committee noted that the source organism used to produce two of the enzymes used in trehalose production did not have a history of food use. Further evidence was sought from the applicant to provide reassurance that this organism could not produce toxins under the conditions of the fermentation process used to produce the two enzymes, that might be carried through into the final food ingredient. Clarification was sought regarding the quality controls for the production of the enzyme preparations, and the batch to batch purity of the Trehalose product. These additional data were provided by the applicant.

The Committee agreed that the application contained good specification data and a detailed description of the production process. The process is well controlled and a consistent product is produced. A specification for trehalose produced by this enzymatic process has been agreed by the Food and Agriculture Organisation, as part of an evaluation by the joint FAO and World Health Organisation (WHO) Expert Committee on Food Additives (JECFA).

There are no nutritional concerns for the product as trehalose is readily converted to glucose. The eating occasion data provided show that there was no glucose overload on the occasions when trehalose was consumed.

The trehalose produced by this enzymatic process has been shown to be

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Detailed descriptions of underlined words are contained in the Glossary

non-toxic and non-mutagenic. Many of the proposed uses for trehalose are mutually exclusive and there are sufficient safety factors between the predicted intake of the product and the level tested in experiments. There was a 60x safety margin between the proposed average intake of trehalose and the highest dose tested in animals and a 20x safety margin between the proposed extreme (90th percentile) intake and the highest dose tested.

No information was provided in the initial application dossier concerning proposals for labelling of the product. During their deliberations, Members of the ACNFP were concerned that diabetics might be unaware that trehalose was a disaccharide of glucose, and not take it into consideration when managing their dietary calorific intake.

It was therefore suggested that it might be helpful to include the description ‘a sugar’ after the name trehalose in the ingredient list, and the Committee therefore sought the advice of the UK Food Advisory Committee on the labelling requirements for trehalose in relation to the needs of diabetics. Taking account of the advice received, the Committee recommended that trehalose should be listed as an ingredient in the foods to which it is added. In addition, trehalose content should be taken into account when determining nutritional labelling information, particularly the content of sugars and carbohydrates in food products, so that diabetics are fully able to manage their overall calorie intake. The Committee was advised that there were no general powers to add a description such as ‘a sugar’ to the name trehalose in the ingredient list. In addition, under the EC Food Labelling Regulations, the term ‘sugar’ is a reserved generic description that may only be used for ingredients that are ‘any type of sucrose’ and may therefore not be used as a description to accompany trehalose. Furthermore, other materials, such as maltose and lactose, that may also be added to a range of food products, are not described in this way, and thus there is no precedent for such additional information on the labels of foods containing trehalose. Nevertheless, the Committee considers that information should be provided to health professionals caring for diabetics and to the relevant support groups, so that diabetics are aware that trehalose is a source of glucose. This approach has been adopted in the past to ensure that diabetics have an above average knowledge of the nutritional quality of food.

The Committee noted that some of the enzyme preparations used to produce trehalose have not been formally assessed for safety in their own right. However, the Committee was satisfied that the detailed processing information provided, together with the range of toxicological data on trehalose produced using this process, provided sufficient reassurance as to their safety for this particular use. However, the Committee agreed that the applicant should be strongly encouraged to submit, for formal evaluation, information on the enzyme preparations used in the trehalose

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production process that have not yet been evaluated for their general food safety, as soon as possible particularly if other food uses are anticipated.

The Committee opinion on this application was forwarded to the Commission for consideration by other Member States at the beginning of October 2000. A copy of this opinion is at Appendix 2.

2.2 Echium oil

A submission was received from John K King & Sons Ltd for an opinion from the UK Competent Authority, under the EC Novel Food Regulation 258/97, for approval to market Echium Oil. This is a complex triglyceride obtained from the plant *Echium plantagineum* (Purple Vipers Bugloss). The oil is produced by a combination of known extraction techniques used in the production of edible oils suitable for human consumption. It is proposed that Echium oil will be sold to food and health food manufacturers throughout Europe as an alternative to existing oils and fats rich in omega-6 or omega-3 polyunsaturated fatty acids, such as evening primrose oil. However, neither the plant nor its products have hitherto been used for human consumption to a significant degree within the Community.

The Committee considered the application at its November meeting and raised a number of concerns that needed to be addressed to enable a full risk assessment to be carried out. Echium oil is known to have a pharmacological action on skin and Members were concerned that no toxicological or human exposure data had been provided.

Data on the total protein (cytochrome C allergen) levels were also insufficient, and Members considered that a more robust and reliable protein evaluation method would be required before any views could be given. Members also noted that there should be a maximum limit for cyclopropenoid and epoxy fatty acids in the specification

The Committee concluded that the product specification was not supported by sufficient analytical data and there was a lack of information on human exposure. They therefore requested further data be obtained on the above and also on the characterisation of the identity and levels of components in the unsaponifiable fraction.

2.3 PrimaDex – update

This application was described in the 1999 Annual Report[14]. The opinion of the European Scientific Committee for Food was considered at the Standing Committee for Foodstuffs in December 2000 and it was agreed to approve this application.

Detailed descriptions of underlined words are contained in the Glossary

3 Applications submitted to other Member States

3.1 High Pressure Processing

The French Competent Authority had evaluated an application from Danone for the high pressure processing of fruit based preparations and given a favourable opinion. The Committee was asked to comment on the summary of the application and French initial opinion and to consider whether they agreed with the proposed approval.

The Committee sought clarification of a number of points regarding the specifications of the food preparation, quality assurance testing and the process controls. They concluded that in order to protect against botulism, the approval for the use of high pressure treated fruit preparations should be limited only to final products whose characteristics conformed to the recommendations given in the Report on Vacuum Packaging and Associated Processes published by the UK Advisory Committee on the Microbial Safety of Food (ACMSF)[15].

The Committee generally agreed with the opinion of the French Competent Authority and was content for clearance to be given for the fruits listed when processed in the manner described in the application dossier only. The Committee's opinion on this application was forwarded to the Commission in July 2000. A copy of this letter is at Appendix 3.

3.2 Novartis BT11 Sweet Maize

The ACNFP was asked for its views on an opinion from the Netherlands Competent Authority (CA) on an application made under the Novel Foods Regulation for approval of fresh and processed food products derived from a genetically modified (GM), sweet maize (Bt 11). The maize has been modified to confer insect resistance and the Netherlands CA had given this a favourable opinion.

The Committee raised a number of concerns on various aspects of the data. The toxicological data referred to studies from Monsanto with field maize and not the Bt 11 sweet maize in question. Studies on the Bt protein were carried out on material isolated from *E.coli* and not the protein expressed in the plant. Again studies on the expression of the PAT protein were carried out in field maize and not the sweet maize.

There were also concerns on the quality of the molecular biology data, and the Committee sought clarification of the additional unexplained bands that appear on Southern Blots.

The Committees' opinion on this application was forwarded to the European Commission in August 2000. A copy of the letter is at Appendix 4.

Objections were raised by other Member States to the initial assessment for this product and the European Commission therefore asked its Scientific Committee for Food (SCF) for an opinion.

At the time of going to press the SCF had not delivered its opinion on this application.

3.3 Monsanto GM Maize

The ACNFP was asked for its opinion on an application made under the Novel Foods Regulation 258/97 to the Netherlands Competent Authority (CA) for approval of food and food ingredients derived from GM maize tolerant to the herbicide glyphosate.

The Committee considered that further information was required before it could deliver an opinion on the validity of the applicant's conclusions regarding the lack of allergenicity/toxicity of the modified EPSPS gene.

A copy of the letter to the Commission seeking clarification on the above is attached at Appendix 5.

Other Member States also raised objections to the initial assessment for this product and the European Commission therefore asked its Scientific Committee for Food (SCF) for an opinion. The SCF gave a favourable opinion in October 2000.

At the time of going to press the SCF opinion had not yet been considered by the Standing Committee for Foodstuffs.

3.4 Phytosterol Esters – update

The UK views on this application were described in the 1999 Annual report[14]. Objections had been raised by a number of other Member States to the initial opinion expressed by the Netherlands Competent Authority on the application for approval of phytosterols for use in yellow fat spreads. The application was therefore considered by the European Commission's Scientific Committee for Food, who issued a favourable opinion. This opinion was voted on by the Standing Committee for Foodstuffs in June 2000, and it was agreed to approve the application. A decision from the Commission was published on the 24th July 2000. This included a requirement for labelling that the product is not nutritionally appropriate for

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pregnant and nursing women, and young children. This requirement meets the concerns expressed by the ACNFP.

A copy of the Commissions decision can be found on their website at: *www.europa.eu.int/eur-lex/en/lif/dat/2000/en_300D0500.htm*

3.5 GM *Radicchio rosso*/Green Hearted Chicory – update

This application was described in the 1998[13] and 1999[14] Annual Reports. An opinion is still awaited from the European Commission Scientific Committee for Foods.

3.6 Nangai nuts – *Canarium indicum* L (France) – update

In 1999 the ACNFP was asked for its opinion on an application made to the French Competent Authority (CA) to determine whether a history of safe use outside the Community provided sufficient reassurance for the safe consumption of Nangai nuts in Europe.

The Committee was concerned that other members of the *Canarium* family are know to contain intrinsically toxic substances and that the history of safe use in the Pacific region did not provide sufficient reassurance for consumers in the Community. The Committee therefore requested further information on this and number of other concerns that they raised (see 1999 ACNFP Annual Report[14]).

Other Member States raised similar concerns and the European Commission requested advice from its Scientific Committee for Food (SCF). The SCF published its opinion on 8 March 2000 and the applicant was requested to submit further information in respect of the concerns raised. Since the information was not provided a decision was taken by the Commission at the Standing Committee for Foodstuffs, on 19 December 2000, to reject the application. A copy of the Commissions decision can be found on their website at: *www.europa.eu.int/eur-lex/en/if/dat/2001/en_301D0017.htm*

4 Other issues considered by the ACNFP

Decisions on Novel Food Status

4.1 Lyprinol

In 1999 the Committee considered an enquiry from Bodycare Corporation Pty Limited about the regulatory status of their product “Lyprinol”. This is a marine oil derived from freeze-dried powder of the New Zealand Green Lipped mussel – *Perna canaliculus* (see 1999 Annual Report[14)].

On the basis of the information received, it appeared that Lyprinol might fall within the scope of the EC Novel Foods Regulation[1] and therefore the Secretariat sought guidance from the Commission regarding the status of this product.

Meanwhile the Committee requested further information on the production quality controls, likely intakes and potential changes in fatty acid composition. Further information on these aspects supplied by the company was considered at the ACNFP’s meeting in July 2000. Although this offered some reassurance, it was not considered sufficient to satisfy the Committees concerns. However, it was felt that before these issues are taken any further, with the company, guidance on the regulatory status of the product should be obtained from the Commission.

4.2 Revised specification for myco-protein

Myco-protein produced by RHM (Marlow Foods) had been cleared for food use in the UK following consideration under the voluntary assessment scheme in 1983.

In 2000, the Committee was asked to advise whether, on the basis of new information presented by the Company, it was satisfied that the RNA composition of the myco-protein could be allowed to increase from 2% to 6% as a result of a change in the production conditions without compromising the safety of the product. Members were also asked whether or not the proposed change in specification would require re-approval of myco-protein under the novel food regulations.

The Committee’s view was that the myco-protein with an increased RNA content would not require approval under the Novel Food Regulation but recognised that ultimately this was a decision for the EC Standing Committee on Foodstuffs. Before they could give an opinion on the safety

of the product the ACNFP requested additional consumption data in order to assess the impact of intake of increased levels of RNA for groups such as vegetarians.

The company provided some further data for scrutiny by this Committee, however this did not include detailed data on intakes by vegetarians. The initial limit on RNA in the specification for myco-protein had been set to restrict purine intake. The ACNFP noted that other sources of protein in the diet that would be replaced by myco-protein also contained purines, and therefore requested that the Secretariat seek further data on the intakes of purines from these other protein sources. In this way, the revised specification for Myco-protein, could be considered in the context of overall purine intake from existing dietary items. These further data were obtained using the FSA Consumer Exposure Team (CERT) Dietary Exposure Assessment Programme, which was set up to give the mean daily purine intake levels from a wide range of foodstuffs.

Members were concerned that the threefold increase in the levels of purine could increase the uric acid levels in the blood of those consuming myco-protein and lead to an increase in cases of acute gout in certain sections of the population, in particular adult males.

Having considered all the data available Members concluded that this revision to the specification should not be cleared without detailed feeding studies on the effects of the revised product in certain sections of the population. These studies should include the effect of ingestion of myco-protein (6% RNA) on the excretion of uric acid and also information on the average myco-protein consumption of vegetarians. The Company was informed of these comments.

Reports and other issues

4.3 Human Studies and Taste Trials

In 1992, the ACNFP published guidelines on the conduct of taste trials of novel foods (including Genetically Modified (GM) Food) using human volunteers. The Committee decided to revise these guidelines to reflect the developments since 1992 and the introduction of the EC Novel Foods Regulation[1] in 1997. The guidelines relate to both the ethical and safety criteria that need to be taken into consideration in performing such trials.

In addition to the above guidelines, the ACNFP also decided to produce guidance that addresses the broader issues involved when conducting studies with human volunteers on novel foods. The aim of these was to review briefly the role of human studies in the pre-market safety

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assessment of novel foods. The focus is on the circumstances in which such studies might be appropriate and the issues (including ethical and study design and protocols) that need to be considered when conducting such studies for this purpose.

4.4 Cholesterol lowering spreads

Issues arising in relation to the labelling of foods containing Benecol were described in the 1999 Annual Report[14]. Members were informed that a number of issues relating to cholesterol-lowering food ingredients in general were discussed by the Novel Food Competent Authorities during 2000. These included the desirability of labelling on the food products themselves and whether, if such labelling is required for novel foods, this should be extended to foods containing Benecol. This is a substance whose use fell outside the terms of the Novel Food Regulation as it had been on sale in Finland prior to May 1997 but which was nevertheless new to consumers in many other Member States including the UK.

Members were also informed that the Food Advisory Committee (FAC) had had a further discussion of a number of issues relating to cholesterol-lowering food ingredients in 2000, including the desirability of labelling on the food products themselves. Members were informed that the FAC had re-affirmed its initial advice that these products should be labelled.

4.5 Safety consideration of unauthorised GM ingredients

The Committee was asked to consider whether the presence of two unauthorised GM ingredients allegedly present in tortilla chips on sale in the UK was a concern for human health. A study commissioned by Friends of the Earth claimed that ingredients derived from maize lines GA21 and DBT418 had been found in tortilla chips. Neither variety has clearance for food use in Europe although both are approved for food use in the US. The analysis reported by Friends of the Earth showed that the level of any GM material was exceedingly small. At the levels reported the Committee concluded that there was no threat to human health. Subsequent tests found no unauthorised GM ingredients. Two statements were issued by the Food Standards Agency on 14 November and 20 December which reflected the advice of the Committee.

These statements can be found on the FSA website at:
www.foodstandards.gov.uk/farm_fork/gm_food.htm

4.6 Monsanto Soya Beans

In May Monsanto provided the ACNFP with further data regarding molecular characterisation of their Roundup Ready soya bean line that had originally been cleared by the ACNFP in 1994. This has primarily involved additional sequencing to determine the flanking sequences at either end of the '35S promoter transit peptide-EPSPS gene Nos terminator' insert. The insert was introduced into the transformation vector as a *Hind* III fragment. Sequencing the 5' and 3' ends of the insert has revealed no additional sequences upstream of the 35S promoter but has identified the presence of a 250bp partial fragment of the EPSPS gene downstream of the Nos terminator. A more sensitive hybridisation procedure has also demonstrated the presence of a separate 72bp EPSPS fragment. The additional fragments were confirmed as having been introduced in the original transformation event and are present in all lines derived from the original transformation event. Northern blot analysis demonstrated that only the intact EPSPS gene is transcribed, as neither the 250 or the 72bp fragments are under the control of regulatory sequences. Similarly Western blot analysis demonstrated that only the intact full-length fragment is translated. This work was documented in a Monsanto report including a safety assessment evaluation, and the data considered by a panel of experts.

The Committee considered these data and requested the sequence of the 72bp fragment and its flanking sequences, and information regarding translation of this sequence in the event that a fused protein might result from insertion of this fragment, and comparison of this sequence with those in the protein databases. The Committee also requested bioinformatics data on the target regions without the inserts to address the question of whether or not these regions encode a protein in the parent Soya bean. Experimental data to confirm the results of the previous bioinformatics study including identifying any proteins encoded either side of the 72bp sequence and determining whether or not this region of DNA is transcribed was also requested.

Monsanto supplied all the data requested by the Committee. The ACNFP concluded that they were content with the information provided and that they did not consider these new data altered their original safety assessment of the Soya beans. Two other committees also assessed these data, the Advisory Committee for Releases into the Environment (ACRE) and the Advisory Committee on Animal Feedingstuffs (ACAF) and both were satisfied that the new data did not alter the original safety assessment. The Commission and other Member States were informed of the UK's opinion in September by the Department of the Environment, Transport and the Regions (DETR). In November the Committee was informed of further analysis regarding a further fragment at the 3'end of the

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epsps insert which cannot be attributed to the wild type plant DNA. The Committee has considered a summary of the data and requested that all the data be made available to the Committee in order that it can conclude its discussions.

4.7 Rethinking Risk – The role of Multi-Criteria Mapping (MCM) approach

Members were invited to comment on a report of this procedure published by the University of Sussex, Science and Technology Policy Research Unit in association with GeneWatch. The Committee considered that the approach had merit in ensuring that a wide range of different options were identified which would be of value when deciding on how a particular risk should be addressed. However the risk assessment should remain a separate part of the overall analysis process, reliant solely on the science.

Members considered that the report's recommendations must be viewed with caution as only twelve participants took part in the study. Nevertheless, the Committee welcomed the broader debate stimulated by this report.

4.8 Review of risk procedures

The risk procedures used by the Government's scientific advisory committees were reviewed by the Government Chief Scientific Advisor, Sir Robert May, the Chief Medical Officer, Liam Donaldson and the Chairman of the Food Standards Agency, Sir John Krebs during 2000. A report of the review was sent to all the Members in August for comment and for action to be taken in response to its recommendations. Professors Bainbridge and Woods had given evidence to the review group as Chairpersons of the ACNFP and COT.

Members found the report a useful compilation of best practices and noted that a lot of the information given in the report came from the work of the ACNFP. Members agreed that the role of the Committees was to provide scientific advice, which would be used by Government, together with other advice, in the formation of policy. The Committee had a role to play in communicating this scientific advice although this fell mainly to the Chairman rather than individual Members.

It would be helpful if clarification could be given on the situations in which committees might usefully put forward possible policy options, and those in which this approach would not be appropriate. In any situation it is clear that it is the role of Government to consider the possible policy options and to decide on a way forward.

The ACNFP agreed that the advisory committees should be as open as possible at all stages of the risk assessment process, whilst noting the need to operate within commercial confidentiality constraints.

4.9 Code of Practice for Scientific Advisory Committees

The Office of Science and Technology issued a consultation document on a Code of Practice for Scientific Advisory Committees that was sent to all the members of the ACNFP in the summer. Members were asked for their views on the consultation document so that their comments could be incorporated into the Food Standards Agency response. Again Professors Bainbridge and Woods were involved in discussions with OST.

The main issue discussed in this report was that of openness, and Members were very committed to the principles of openness and transparency, as set out in the draft document. Nevertheless there was a concern that Members of the advisory committees should be properly protected. For instance, comments made by Members are not attributed to individuals in minutes that are published and thus Members are less likely to become targets of personal attacks. There was also the concern that if Members were not properly protected then individuals would be less likely to serve on committees and thus the quality of scientific advice received by Government could be compromised.

Members accepted that they had a role to play in communicating their advice, but were of the view that this should be restricted to scientific issues. Training should be offered to all new Committee Members to enhance communication skills and interaction with the media, although much of the task of communication of the Committee's views would fall on the Chairman, rather than on individual Members. The role of communication of risk management issues was seen as one for the Food Standards Agency and for Government more generally and not for the advisory committees.

Members also considered whether it was feasible or desirable for the ACNFP to move even further towards greater openness than current practices. Members were agreed that it would not be practical for a Committee considering detailed applications for approvals of novel foods to hold all its meetings in public. There were a number of practical considerations, such as how to handle issues relating to confidential commercial data that would need to be resolved before any such moves could be considered. The current practice of publishing application dossiers before meetings, as well as publishing committee papers and the minutes of meetings, provided the public with sufficient information on how decisions were reached and also allowed an opportunity for the public to have an input into the evaluation process. The Members however did agree that ACNFP should hold open meetings to discuss more general issues.

Finally, it was suggested that with the demise of the Consumer Panel, it would be helpful if some other forum could be instituted for the lay members of the scientific advisory committees to meet to discuss common issues and concerns.

4.10 OECD Consensus Document

The Organisation for Economic Co-operation and Development Task Force for the safety of novel foods and feeds is producing a series of consensus documents on various crops. The purpose of these documents is to provide a technical tool for regulatory officials as a general guide and reference source to aid in the safety assessment of modified crops. Two documents, on soya bean and oilseed rape, which were nearing completion were forwarded to the ACNFP for their comments. A further two documents on potatoes and sugar beet which are at a much earlier preparative stage were also considered by the Committee. The Committee welcomed the development of these documents and felt that they would provide a useful source of information.

Comments on the documents were forwarded to the OECD Task Force.

4.11 OECD response to G8 Communiqué

As part of the OECD's response to a request from the June 1999 G8 summit an international conference was organised in Edinburgh. Several members of the Committee attended the conference on the scientific and health aspects of genetically modified foods "GM food safety: facts, uncertainties and assessment". The report of the conference and other reports sent by the OECD to the July 2000 G8 summit can be found on the OECD website at:
http://www.OECD.org/subject/biotech/g8_docs.htm

4.12 Codex Task Force

The first meeting of the Codex Task Force on foods derived from biotechnology took place in March 2000. The task force agreed to develop two key documents: a set of principles for the risk analysis of foods derived from modern biotechnology and accompanying guidelines for the safety assessment of genetically modified foods. These documents were considered by the Committee at its 47th meeting held on 16 November 2000.

The Committee welcomed the opportunity to comment on the documents and asked to be kept informed of their progress.

5 Other activities

5.1 Openness

For a number of years the ACNFP has been taking steps to ensure the transparency of the assessment process for novel (including GM) foods. In 2000 the Committee has continued to make available as much information as possible in advance of its meetings. This includes meeting agendas and Secretariat papers laid before the Committee, as well as the placing of novel food marketing applications, excluding commercially confidential data, on its website for public comment prior to discussion. In addition to these measures, the Committee continues to encourage companies that make novel food applications to deposit a copy in the British Library.

The two full novel food applications received in 2000 were the first under the new openness procedures introduced in December 1999 and both were placed on the ACNFP website for public comment. They can still be found there for information, along with other details about how applications are considered and how to make comments on future applications. For convenience, it is possible to leave an expression of interest on the website whereby you will be informed by e-mail when a new application is placed there for comment. For information, the legal basis for the publication of novel food applications has changed, even though the procedure remains the same as published in the previous annual report and on the ACNFP website. In summary, on 21 December 1999 measures came into effect amending the UK Novel Foods Regulations, insofar as they apply to England, making provision for the ACNFP to make public all non-confidential information submitted to it as part of an application to market a novel, including GM, food in the UK. Ministerial Directions issued on this date required this information to be disclosed in accordance with accompanying Guidance Notes on confidentiality. When the Food Standards Agency was established in April 2000, Section 19(1)c of the Foods Standards Act gave the Agency the powers to publish all information subject to the requirements of the Data Protection Act. This replaced the measures introduced in December 1999, however, the Guidance Notes accompanying the Ministerial Directions issued then have been adopted by the Agency as a statement of how it proposes to deal with novel food applications in relation to disclosure.

This year the Committee reconfirmed its commitment to holding open meetings when generic issues are to be discussed, although it was agreed that issues such as how to handle the discussions of confidential data

supporting applications needed to be resolved before the Committee meetings could be held in public. Nevertheless, the minutes of such meetings and related papers continue to be published.

The Committee has also published general corporate information on its work and its members through its website, corporate brochure and annual report.

All of this and further information can be found on the ACNFP's area of the FSA website at www.foodstandards.gov.uk/committees/acnfphp.htm and from the ACNFP Secretariat.

5.2 R&D Reports

The Committee considered two completed R&D reports, which had been funded as part of the research programme on the safety of novel foods. The projects considered were commissioned when responsibility of the programme fell to MAFF. The final reports for these projects have been placed in the MAFF library.

Project RG0214 titled 'Risk assessment of genetically modified organisms in the agricultural environment' was carried out at the University of Manchester. The overall aim of the project was to address the issue of horizontal gene transfer by studying the potential movement of mobile genetic elements between unrelated species. The initial aim was to identify and isolate mobile genetic elements of prokaryotic origin in plant genomic DNA. Using PCR, no evidence was obtained to suggest that such elements are present in plant genomic DNA. A phylogenetic analysis of a mariner element, a transposable element first identified in *C. elegans*, showed good evidence for horizontal transfer across species raising the question as to whether other transposable elements are equally as widespread.

Project FS0219 titled 'Persistence and potential infectivity of live bacteria in foods' was carried out at the Institute for Food Research, Norwich. The overall aim of the project was to investigate the presence of virulence determinants in food and pathogenic isolates of *Enterococcus* and the potential for acquisition of virulence determinants by non-pathogenic *Enterococcus* strains through conjugation with virulent strains. Seven *Enterococcus* virulence genes were identified from a collection of 141 food and pathogenic *Enterococcus* strains. These strains consisted of dairy starter strains, food isolates and medical isolates. It was also shown that the transfer of virulence determinants to non-virulent isolates of *Enterococcus* is feasible. Such results may be significant in the rapidly expanding probiotic market where the use of such *Enterococcus* strains should be carefully monitored.

Detailed descriptions of underlined words are contained in the Glossary

5.3 Research Programme (G02) – The safety assessment of GM foods

The Food Standards Agency will launch a major new research programme in April 2001, which will investigate how new and emerging techniques can be applied to the safety assessment of novel and GM foods. The Agency hosted a workshop at the end of November to discuss the current status of the technology and how we might use it in the safety assessment process. The output from the workshop formed the basis of the tender for research proposals, which was published on 14 December. A number of Committee Members attended the workshop.

6 Developments elsewhere

6.1 EC Directives on Food Irradiation

In February 1999 the European Council and the European Parliament published two EC Directives (1999/2/EC[16] and 1999/3/EC[17]) on foods and food ingredients treated with ionising radiation in the Official Journal of the European Communities.

- Directive 1999/2/EC[16] lays down the general provisions such as the conditions for treatment and the rules governing the approval and control of irradiation, as well as changing the rules on the labelling of foodstuffs that have been treated with ionising radiation.

- Directive 1999/3/EC[17] establishes an initial positive list of foodstuffs that can be treated with ionising radiation and freely traded across the whole European Community.

These Directives came into effect on 20 September 2000.

6.2 GM Labelling Update

EC Regulation 49/2000[19] came into force on 10 April 2000. The Regulation amends EC Regulation 1139/98[18] (on the labelling of foods containing GM Soya and maize). It extends the scope of the labelling requirements to include foods sold to catering establishments, and introduces a 1% *de minimis* threshold for the adventitious contamination of non-GM produce. The aim of the 1% threshold is to ensure that food ingredients obtained from non-GM sources do not need to be labelled as GM if they contain low levels of GM material as a result of adventitious contamination – this flexibility does not apply to supplies obtained from sources of unknown origin. The new Regulation also makes clear that steps should be taken to keep the level of adventitious contamination to a minimum. It is important to note that the 1% level applies to Soya and maize ingredients and not the final food; the level in the final food will be much lower.

EC Regulation 50/2000[20] also came into force on 10 April 2000. This Regulation requires the labelling of foods and food ingredients containing additives and flavourings containing DNA or protein resulting from genetic modification, and therefore makes such additives and flavourings subject to

Detailed descriptions of underlined words are contained in the Glossary

the same labelling rules as those of the Novel Foods Regulation (258/97)[1]. The Commission has undertaken to bring forward a proposal to set a *de minimis* threshold for additives in due course.

The Genetically Modified and Novel Food (Labelling) (England) Regulations 2000[21] also came into force on 10 April 2000 in support of the new EC Regulations. The domestic Regulations make provision for the continued enforcement in England of existing rules on GM labelling (i.e. EC Regulation 1139/98[18] (as amended by EC Regulation 49/2000[19]) and Article 8(1) of EC Regulation 258/97) and provide for the enforcement in England of two new EC Regulations (49/2000[19] and 50/2000[20]). The domestic Regulations also provide flexible labelling arrangements for businesses selling foods loose or pre-paid for direct sale. This includes foods sold in restaurants, take-aways and delicatessens. Similar domestic Regulations apply in Scotland and Wales. Legislation will follow in Northern Ireland shortly.

7 Contact points

For further information about the general work of the Committee or about specific scientific points concerning individual submissions (which have been made or are being made) contact in the first instance:

Mrs Sue Hattersley
ACNFP Secretary
Room 526B
Aviation House
125 Kingsway
London
WC2B 6NH

Tel (switchboard): 020 7276 8000
Tel (Direct line): 020 7276 8565
Fax (Direct line): 020 7276 8564

The FSA Website can be found at *http:\\www.foodstandards.gsi.gov.uk.*
Information can also be requested via e-mail at:
acnfp@foodstandards.gsi.gov.uk.

8 References

1. European Commission. Regulation (EC) No 258/97 of the European Parliament and of the Council concerning novel food and novel food ingredients. *Official Journal of the European Communities*, No L43 of 14th February 1997.

2. Commission of the European Communities 1997: Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No. 258/97 of the European Parliament and of the Council. C(97) 2634 final. *Official Journal of the European Communities* Vol. 40 L253/1-45 16 September 1997.

3. UK Food Safety Act (1990). ISBN No. 0 –10-541690-8

4. Advisory Committee on Novel Foods and Processes. *Annual Report 1989*. Department of Health and Ministry of Agriculture, Fisheries and Food, 1990. (Available from the ACNFP Secretariat).

5. Advisory Committee on Novel Foods and Processes. *Annual Report 1990*. Department of Health and Ministry of Agriculture, Fisheries and Food, 1991. (Available from the ACNFP Secretariat).

6. Advisory Committee on Novel Foods and Processes. *Annual Report* 1991. Department of Health and Ministry of Agriculture, Fisheries and Food, 1992. (Available from the ACNFP Secretariat).

7. Advisory Committee on Novel Foods and Processes. *Annual Report 1992*. Department of Health and Ministry of Agriculture, Fisheries and Food, 1993. (Available from the ACNFP Secretariat).

8. Advisory Committee on Novel Foods and Processes. *Annual Report 1993*. Department of Health and Ministry of Agriculture, Fisheries and Food, 1994. (Available from the ACNFP Secretariat).

9. Advisory Committee on Novel Foods and Processes. *Annual Report 1994*. Department of Health and Ministry of Agriculture, Fisheries and Food, 1995. (Available from the ACNFP Secretariat).

10. Advisory Committee on Novel Foods and Processes. *Annual Report 1995*. Department of Health and Ministry of Agriculture, Fisheries and Food, 1996. (Available from the ACNFP Secretariat).

11. Advisory Committee on Novel Foods and Processes. *Annual Report 1996*. Department of Health and Ministry of Agriculture, Fisheries and Food 1997. (Available from the ACNFP Secretariat).

12. Advisory Committee on Novel Foods and Processes. *Annual Report 1997*. Department of Health and Ministry of Agriculture, Fisheries and Food 1998. (Available from the ACNFP Secretariat).

13. Advisory Committee on Novel Foods and Processes. *Annual Report 1998*. Department of Health and Ministry of Agriculture, Fisheries and Food 1999. (Available from ACNFP Secretariat).

14. Advisory Committee on Novel Foods and Processes. *Annual Report 1999*. Department of Health and Ministry of Agriculture, Fisheries and Food 2000. (Available from the ACNFP Secretariat).

15. Advisory Committee on the Microbial Safety of Food. *Report on Vacuum packaging and Associated Processes*. HMSO 1992. ISBN 0-11-321558-4 (£6.50)

16. Directive 1999/2/EC of the European Parliament and of the Council on the approximation of the laws of the Member States concerning foods and food ingredients treated with ionising radiation. *Official Journal of the European Communities* L66/16-23 13 March 1999.

17. Directive 1999/3/EC of the European Parliament and of the Council on the establishment of a Community list of foods and food ingredients treated with ionising radiation. *Official Journal of the European Communities* L66/24-25 13 March 1999.

18. Council Regulation (EC) No 1139/98 concerning the compulsory indication of the labelling of certain foodstuffs produced from genetically modified organisms of particulars other than those provided for in Directive 79/112/EEC. *Official Journal of the European Communities*, L159/4-7, 3 June 1998.

19. Commission Regulation (EC) No 49/2000 amending Council Regulation (EC) No 1139/98 concerning the compulsory indication on the labelling of certain foodstuffs produced from genetically modified organisms of particulars other than those provided for in Directive 79/112/EEC. *Official Journal of the European Communities* L6 Vol43/13-14 11January 2000.

20. Commission Regulation (EC) No 50/2000 on the labelling of foodstuffs and food ingredients containing additives and flavourings that have been genetically modified or have been produced from genetically modified organisms. *Official Journal of the European Communities* L6 Vol43/ 15-17 11 January 2000.

21. SI 2000 No 768 – The Genetically Modified and Novel Foods (Labelling) (England) Regulations 2000. ISBN 0-11-099029-3 (£2.50)

9 Glossary

Allergen: a substance to which an individual is hypersensitive and which causes an allergic response.

Allergenicity: a potential or ability to illicit an allergic response.

Bioinformatics: the use of computers in solving information problems in life sciences, it involves the creation of an extensive database on genomes, protein sequences etc, followed by 3D modelling of biomolecules and biological systems.

Conjugation: the union of 2 unicellular organisms, or the male and female gametes of multicellular organisms, in order that genetic material is exchanged, followed by partition.

Cyclopropenoid: a type of fatty acid.

De minimis threshold: the threshold of 1% for the adventitious contamination of non-GM produce.

Disaccharide: a carbohydrate composed of 2 sugar molecules.

Eating occasion: points in the day when the food ingredient was consumed, particularly when at a single point rather than when consumption is spread over several meals.

Enterococcus: streptococcus bacteria present in the intestinal tract.

Epoxy fatty acids: a fatty acid containing an epoxy group (an oxygen atom bound to two linked carbon atoms).

Gout: a disease with inflammation of the smaller joints as a result of excess uric acid salts in the blood.

Hybridisation: formation of a hybrid. Composed of 2 or more portions of DNA from different origins.

Ionising radiation: a form of radiation with sufficient energy to cause an atom to lose or gain one or more electrons leaving it electrically charged. A charged atom is referred to as an ion, hence the term ionising radiation.

Pathogenic: causing disease.

Pharmacological: relating to the composition, properties and action of medicinal drugs or other biologically active chemicals.

Phylogenetic: relating to the evolutionary development of any plant or animal species. The ancestral history of an individual.

Polyunsaturated: fatty acids with more than one double bond in their hydrocarbon chain.

Prokaryotes/Prokaryotic: unicellular organism whose small, simple cells lack a membrane-bound nucleus, mitochondria, chloroplasts and other membrane bound organelles. Their DNA is in the form of a single cellular molecule not complexed with histones.

Proteinaceous: composed of protein.

Purine: type of nitrogenous organic base, of which adenine and guanine are the most common in living cells, occurring in nucleic acids where they pair with pyrimides. When linked with ribose or deoxyribose phosphates form nucleotides.

Transgenic: animals or plants that have had genes artificially introduced by genetic modification.

Triglyceride: a compound consisting of a glycerol molecule esterified at each of its three hydroxyl groups by a fatty acid group.

Appendix I

ACNFP – remit, membership and list of members' interests, code of conduct and interactions with other committees

Remit

The Advisory Committee on Novel Foods and Processes is an independent body of experts whose remit is:

> *"to advise the central authorities responsible, in England, Scotland, Wales and Northern Ireland respectively on any matters relating to novel foods and novel food processes including food irradiation, having regard where appropriate to the views of relevant expert bodies".*

Officials of the Food Standards Agency provide the Secretariat. As well as formal meetings, the Committee organises workshops on specific topics related to its remit.

The interaction between the ACNFP and other independent advisory committees is outlined in Figure 1.

Membership and Members' Interests

The membership of the Committee provides a wide range of expertise in fields of relevance in the assessment of novel foods and processes. A list of the membership during 2000, together with the names of the FSA assessor and the Secretariat may be found overleaf.

In common with other independent advisory committees the ACNFP is publishing a list of its members' commercial interests. These have been divided into different categories relating to the type of interest:–

Personal:–
a) direct employment or consultancy;
b) occasional commissions;
c) share holdings.

Non-personal:–
a) fellowships;
b) support which does not benefit the member directly e.g. studentships.

Details of the interests held by Members during 2000 can be found on page 30

A copy of the code of conduct for ACNFP Members can be found on page 33

MEMBERSHIP OF THE COMMITTEE DURING 2000

Chairman

Professor Janet Bainbridge, BSc, PhD, GradCertEd (Tech), MiBiol, CBiol, SOFHT
School of Science and Technology
University of Teesside, Middlesborough (from September 1997)

Members

Professor P J Aggett, MSc, MB, ChB, FRCP (Lond, Edin & Glasg), DCH
(term of office expired in August 2000)
Head of Lancashire Postgraduate School of Medicine and Health

Professor P Dale BSc PhD, CBiol, MIBiol
Research Group Leader, Genetic Modification and Risk Assessment,
John Innes Centre, Norwich.
Honorary Reader of the University of East Anglia

Professor M J Gasson, BSc, PhD
Head, Department of Genetics and Microbiology
Institute of Food Research, Norwich

Dr J Heritage, BA, D.Phil, C.Biol, MIBiol
Division of Microbiology
School of Biochemistry and Molecular Biology
University of Leeds

Professor D A Ledward, MSc, PhD, FIFST
(term of office expired in August 2000)
Professor of Food Science, University of Reading

Reverend Professor M Reiss BSc, MA, PhD, FIBiol
Senior Lecturer, Homerton College, University of Cambridge

Mrs E Russell Dip.comp (open), BSc
Consumers Representative

Professor I Rowland, BSc, PhD
Director, Northern Ireland Centre for Diet and Health
University of Ulster, Coleraine

Professor T Sanders, BSc, PhD, DSc
Head of Department of Nutrition and Dietetics
Kings College, London

Professor H Sewell MB ChB, BDS, MSc, PhD, FRCP (Lond & Glas), FRCPath, FMedSci
Head of Immunology, Faculty of Medicine and Health Science, University Hospital Medical School, Nottingham.

Dr N A Simmons FRC Path, FIFST
Emeritus Consultant in Microbiology
Guy's & St Thomas' Hospital Trust, London

Professor J O Warner MB, ChB, DCH, MRCP, MD, FRCP, MRCPCH, FRCPCH.
Professor of Child Health, University of Southampton.

Professor R Walker PhD, CChem, FRSC, FIFST
(term of office expired in August 2000)
Professor of Food Science, University of Surrey

Professor H F Woods BSc, BM BCh, DPhil, Hon.FFOM, FIFST, FFPM, FRCP(Lond & Edin)
Sir George Franklin Professor of Medicine, Division of Molecular and Genetic Medicine, University of Sheffield

FSA Assessors

Dr J Bell	Food Standards Agency
Mrs J Whinney	Food Standards Agency (Wales)
Mrs C Wood	Food Standards Agency (Scotland)
Mr G McCurdy	Food Standards Agency (Northern Ireland)

	Personal Interest		**Non-Personal Interest**		**Partner Interest**
Member	**Company**	**Interest**	**Company**	**Interest**	
Professor J Bainbridge (CHAIRMAN)	None	None	Various	Departmental commissioned research and student placements	None
Professor M J Gasson (DEPUTY CHAIRMAN)	None	None	Various	Departmental commissioned research	None
Professor P J Aggett	Nestec, Wyeth	Ad hoc consultancy	Nestec, Milupa, Nutricia, Wyeth Ajinomoto FDF Unilever	Departmental commissioned research and consultancies	None
Dr P Dale	None	None	European Community DETR FSA/MAFF/DETR Research	Occasional Advisor Call off consultant Seed Samples obtained from various companies	None
Dr J Heritage	None	None	None	None	None
Professor D A Ledward	None	None	Various	Departmental teaching & research funded by various food companies	None
Reverend Professor M Reiss	None	None	None	None	None
Professor I Rowland	Colloids Naturels International (CNI) Rouen, France	Consultancy	Coca Cola Kelloggs Sugar Bureau Meat and Livestock Commission Scotia Lipidteknik/ Scotia Pharmaceuticals Cultor Foods Howard Foundation Orafti	Research Research Research Research Research Research Research/ Consultancy Research Research	None

	Personal Interest		**Non-Personal Interest**		**Partner Interest**
Member	**Company**	**Interest**	**Company**	**Interest**	
Mrs E Russell	The Boots Company PLC	Shareholder	None	None	Husband Chief Executive of The Boots Company plc.
Professor T Sanders	Nutrasweet AG	Consultancy	Unilever	Free supply of oils & fats for research purposes	None
	Seven Seas Limited	Consultancy	Cultor Food Science	Research grant	
	ILSI Europe	Fee Paid Work			
Professor H Sewell	None	None	None	None	None
Dr N Simmons	Food Micro Limited	Director and Shareholder	None	None	None
	Infection Management Ltd	Advisor and Shareholder			
	Marks & Spencer plc	Independent Advisor			
	McDonalds Restaurants Ltd	Independent Advisor			
	PPP/ Columbia Healthcare Ltd	Consultant			
	Waitrose Ltd	Independent Advisor			
	Worshipful Company of Fishmongers	Bacteriologist			

	Personal Interest		**Non-Personal Interest**		**Partner Interest**
Member	**Company**	**Interest**	**Company**	**Interest**	
Professor R Walker	Coffee Science Info Centre	Consultancy	None	None	None
	Numico	Consultancy			
	Colloids	Consultancy			
	Naturel International				
	Nestec	Fee			
	Borex Europe Ltd	Consultancy			
	Holland Sweetners	Fee			
	Xyrofin	Fee			
	Tate and Lyle Specialty Sweetners	Fee			
	Hoffman-La Roche	Fee			
Professor J O Warner	ILSI Europe		UBC Pharma Merck	Research Research	None
Professor H F Woods	HSBC	Shareholder	The University of Sheffield receives support from a wide range of national and international food and chemical companies	Trustee of the Harry Bottom Charitable Trust and Special Trustees for the former United Sheffield Hospitals	None
	Halifax Bank	Shareholder			

A CODE OF CONDUCT FOR MEMBERS OF THE ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESS (ACNFP)

Public service values

The members of the ACNFP must at all times

- observe the highest standards of impartiality, integrity and objectivity in relation to the advice they provide and the management of this Committee;

- be accountable, through the Board of the Food Standards Agency and DH Ministers, to Parliament and the public for its activities and for the standard of advice it provides.

The Ministers of the Department of Health are answerable to Parliament for the policies and performance of this Committee, including the policy framework within which it operates.

Standards in Public Life

All Committee members must

- follow the Seven Principles of Public Life set out by the Committee on Standards in Public Life (Annex 1);

- comply with this Code, and ensure they understand their duties, rights and responsibilities, and that they are familiar with the function and role of this Committee and any relevant statements of Government policy. If necessary members should consider undertaking relevant training to assist them in carrying out their role;

- not misuse information gained in the course of their public service for personal gain or for political purpose, nor seek to use the opportunity of public service to promote their private interests or those of connected persons, firms, businesses or other organisations; and

- not hold any paid or high profile unpaid posts in a political party, and not engage in specific political activities on matters directly affecting the work of this Committee. When engaging in other political activities, Committee members should be conscious of their public role and exercise proper discretion. These restrictions do not apply to MPs (in those cases where MPs are eligible to be appointed), to local councillors, or to Peers in relation to their conduct in the House of Lords.

Role of Committee members

Members have collective responsibility for the operation of this Committee. They must:

- engage fully in collective consideration of the issues, taking account of the full range of relevant factors, including any guidance issued by the Food Standards Agency or the responsible Minister;

- in accordance with Government policy on openness, ensure that they adhere to the Code of Practice on Access to Government Information (including prompt responses to public requests for information); agree an Annual Report; and, where practicable and appropriate, provide suitable opportunities to open up the work of the Committee to public scrutiny;

- not divulge any information which is provided to the Committee in confidence;

- ensure that an appropriate response is provided to complaints and other correspondence, if necessary with reference to the sponsor department; and

- ensure that the Committee does not exceed its powers or functions.

Individual members should inform the Chairman (or the Secretariat on his or her behalf) if they are invited to speak in public in their capacity as a Committee member.

Communications between the Committee and the Board of the Food Standards Agency will generally be through the Chairman except where the Committee has agreed that an individual member should act on its behalf. Nevertheless, any member has the right of access to the Board of the Food Standards Agency on any matter, which he or she believes raises important issues relating to his or her duties as a Committee member. In such cases the agreement of the rest of the Committee should normally be sought.

The Chairman of the Food Standards Agency can remove individual members from office if they fail to perform the duties required of them in line with the standards expected in public office.

The role of the Chairman

The Chairman has particular responsibility for providing effective leadership on the issues above. In addition, the Chairman is responsible for

- ensuring that the Committee meets at appropriate intervals, and that the minutes of meetings and any reports to the Board of the Food Standards Agency accurately record the decisions taken and, where appropriate, the views of individual members;

- representing the views of the Committee to the general public; and

- ensuring that new members are briefed on appointment (and their training needs considered), and providing an assessment of their performance, on request, when members are considered for re-appointment to the Committee or for appointment to the board of some other public body.

Handling conflicts of interests

The purpose of these provisions is to avoid any danger of Committee members being influenced, or appearing to be influenced, by their private interests in the exercise of their public duties. All members should declare any personal or business interest which may, or may be *perceived* (by a reasonable member of the public) to, influence their judgement. A guide to the types of interest that should be declared is at Annex 2.

(i) Declaration of Interests to the Secretariat

Members of the Committee should inform the Secretariat in writing of their current personal and non-personal interests, when they are appointed, including the principal position(s) held. Only the name of the company and the nature of the interest are required; the amount of any salary etc. need not be disclosed. Members are asked to inform the Secretariat at any time of any change of their personal interests and will be invited to complete a declaration form once a year. It is sufficient if changes in non-personal interests are reported in the annual declaration form following the change. (Non-personal interests involving less than £1,000 from a particular company in the previous year need not be declared to the Secretariat).

The register of interests should be kept up-to-date and be open to the public.

(ii) Declaration of Interest and Participation at Meetings

Members of the Committee are required to declare any direct interests relating to salaried employment or consultancies, or those of close family members[1], in matters under discussion at each meeting. Having fully explained the nature of their interest the Chairman will, having consulted

[1] Close family members include personal partners, parents, children, brothers, sisters and the personal partners of any of these.

the other members present, decide whether and to what extent the member should participate in the discussion and determination of the issue. If it is decided that the member should leave the meeting, the Chairman may first allow them to make a statement on the item under discussion.

Personal liability of Committee members

A Committee member may be personally liable if he or she makes a fraudulent or negligent statement which results in a loss to a third party; or may commit a breach of confidence under common law or a criminal offence under insider dealing legislation, if he or she misuses information gained through their position. However, the Government has indicated that individual members who have acted honestly, reasonably, in good faith and without negligence will not have to meet out of their own personal resources any personal civil liability which is incurred in execution or purported execution of their Committee functions save where the person has acted recklessly. To this effect a formal statement of indemnity has been drawn up.

THE SEVEN PRINCIPLES OF PUBLIC LIFE

Selflessness
Holders of public office should take decisions solely in terms of the public interest. They should not do so in order to gain financial or other material benefits for themselves, their family, or their friends.

Integrity
Holders of public office should not place themselves under any financial or other obligation to outside individuals or organisations that might influence them in the performance of their official duties.

Objectivity
In carrying out public business, including making public appointments, awarding contracts, or recommending individuals for rewards and benefits, holders of public office should make choices on merit.

Accountability
Holders of public office are accountable for their decisions and actions to the public and must submit themselves to whatever scrutiny is appropriate to their office.

Openness
Holders of public office should be as open as possible about all the decisions and actions that they take. They should give reasons for their decisions and restrict information only when the wider public interest clearly demands.

Honesty
Holders of public office have a duty to declare any private interests relating to their public duties and to take steps to resolve any conflicts arising in a way that protects the public interests.

Leadership
Holders of public office should promote and support these principles by leadership and example.

DIFFERENT TYPES OF INTEREST

The following is intended as a guide to the kinds of interests that should be declared. Where members are uncertain as to whether an interest should be declared they should seek guidance from the Secretariat or, where it may concern a particular product which is to be considered at a meeting, from the Chairman at that meeting. **If members have interests not specified in these notes but which they believe could be regarded as influencing their advice they should declare them**. However, neither the members nor the Secretariat are under any obligation to search out links of which they might *reasonably* not be aware. For example, either through not being aware of all the interests of family members, or of not being aware of links between one company and another.

Personal Interests

A personal interest involves the member personally. The main examples are:

- **Consultancies and/or direct employment** any consultancy, directorship, position in or work for the industry or other relevant bodies which attracts regular or occasional payments in cash or kind;

- **Fee-Paid Work:** any commissioned work for which the member is paid in cash or kind;

- **Shareholdings:** any shareholding or other beneficial interest in shares of industry. This does not include shareholdings through unit trusts or similar arrangements where the member has no influence on financial management;

- **Membership or Affiliation** to clubs or organisations with interests relevant to the work of the Committee.

Non-Personal Interests

A non-personal interest involves payment which benefits a department for which a member is responsible, but is not received by the member personally. The main examples are:

- **Fellowships:** the holding of a fellowship endowed by industry or other relevant body;

- **Support by Industry or other relevant bodies:** any payment, other support or sponsorship which does not convey any pecuniary or material benefit to a member personally, but which does benefit their position or department e.g.:

  (i) a grant for the running of a unit or department for which a member is responsible;

(ii) a grant or fellowship or other payment to sponsor a post or a member of staff or a post graduate research programme in the unit for which a member is responsible (this does not include financial assistance for undergraduate students);

(iii) the commissioning of research or other work by, or advice from, staff who work in a unit for which a member is responsible.

Members are under no obligation to seek out knowledge of work done for, or on behalf of, industry or other relevant bodies by departments for which they are responsible, if they would not normally expect to be informed. Where members are responsible for organisations which receive funds from a very large number of companies involved in that industry, the Secretariat can agree with them a summary of non-personal interests rather than draw up a long list of companies.

- Trusteeships: any investment in industry held by a charity for which a member is a trustee. Where a member is a trustee of a charity with investments in industry, the Secretariat can agree with the member a general declaration to cover this interest rather than draw up a detailed portfolio.

DEFINITIONS

For the purposes of the ACNFP 'industry' means:

- Companies, partnerships or individuals who are involved with the production, manufacture, packaging, sale, advertising, or supply of food or food processes, subject to the Food Safety Act 1990;

- Trade associations representing companies involved with such products;

- Companies, partnerships or individuals who are directly concerned with research, development or marketing of a food product which is being considered by the Committee.

'Other relevant bodies' refers to organisations with a specific interest in food issues, such as charitable organisations or lobby groups.

In this Code 'the Secretariat' means the Secretariat of the ACNFP.

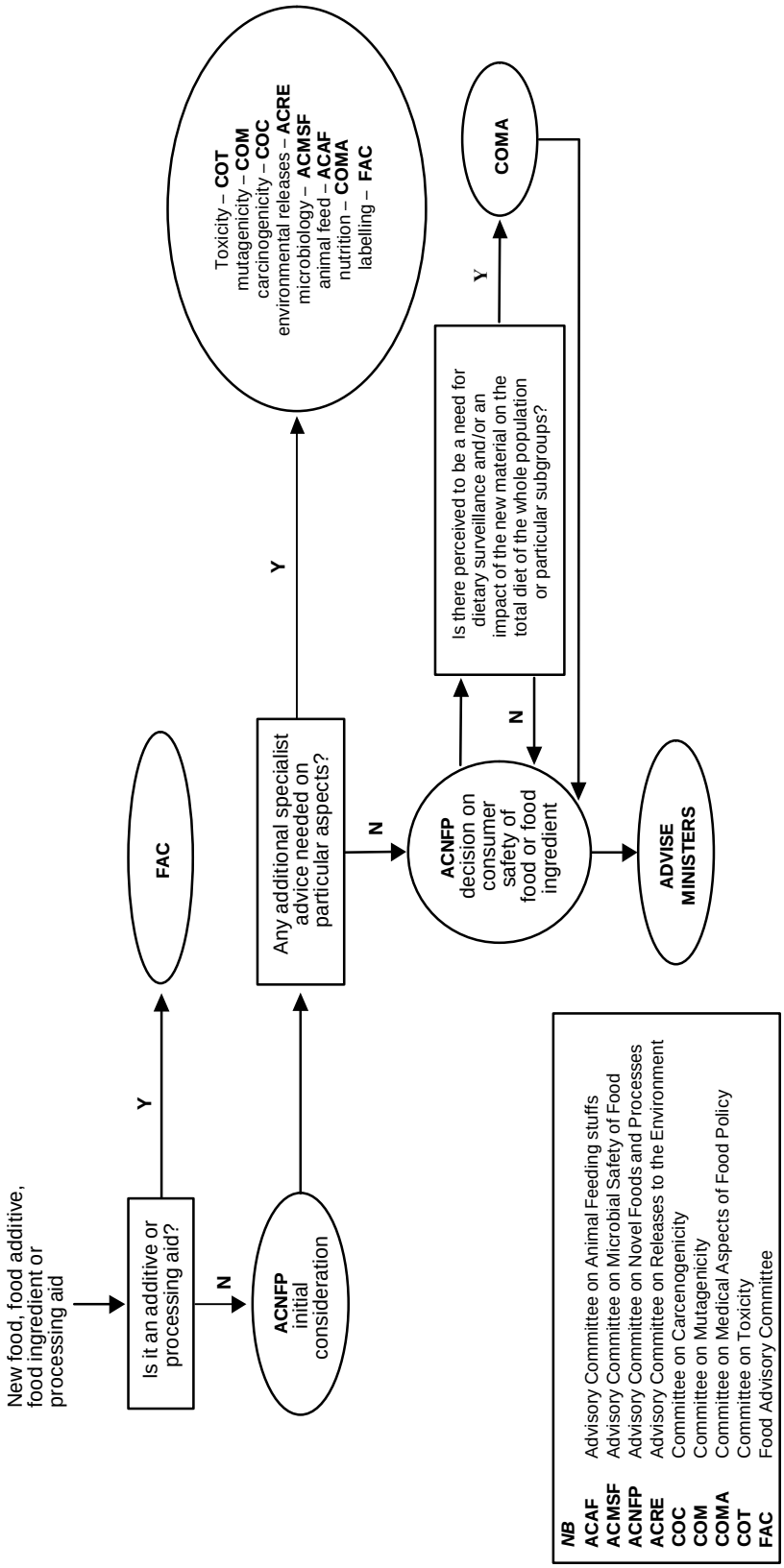


Figure 1: Relationship of ACNFP with other expert committees involved in the assessment of food safety

APPENDIX II

Mr Andreas Klepsch
European Commission
Health and Consumer Protection Directorate General
Rue de la Loi 200
B-1049 Brussels
Belgium Reference:

3 October 2000

Dear Andreas

Initial opinion on Trehalose produced by a novel enzymatic process

The UK received an application for approval of trehalose produced by a novel enzymatic process for approval as a novel food. This application was reviewed by the Advisory Committee on Novel foods and Processes (ACNFP) and their opinion is attached. I apologise for the delay in sending this opinion to you but the evaluation was delayed by the need to seek further detailed information from the applicant.

The UK recommends approval of the application, provided that the trehalose is produced by the process described in the application dossier and that the final trehalose material meets the specification described. We understand that a similar specification was agreed for trehalose produced by this process at the 55th JECFA meeting (2000) and that this specification will be published in due course in FNP 52, add 8 (2000).

I am copying this letter and the UK opinion to other Member States and to the applicant, Dr Albert Bar.

Best wishes

Sue Hattersley
For the UK Competent Authority

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES UK/2000/001

Opinion on an application under the Novel Food Regulation from Bioresco Ltd for clearance of Trehalose produced by a novel enzymatic process

Applicant: **Bioresco Ltd (on behalf of Hyashibara Co Ltd)**

Responsible person: **Dr Albert Bar**

Novel Food: **Trehalose produced by a novel enzymatic process**

EC Classification: **6**

Introduction

1. An application was submitted to the UK Competent Authority on 25 May 2000 by Bioresco Ltd (on behalf of Hyashibara Co. Ltd) for approval of trehalose produced by a novel enzymatic process. In 1990, the Advisory Committee on Novel Foods and Processes (ACNFP), the UK Competent Assessment Body, assessed an application, submitted under the voluntary scheme that then existed in the UK for the assessment of novel foods and processes, for the safety of trehalose extracted from yeast. Trehalose produced from yeast was approved for use in foods (except infant formulae and follow-on formulae) in April 1991, although there is no evidence that this product was subsequently marketed in the Community.

2. Trehalose is a naturally occurring disaccharide that consists of two glucose molecules linked by a 1,1-α-glycosidic bond. Its sweetness relative to that of sucrose is about 40-45%. Hayashibara Co., Ltd. has developed a novel enzymatic process for the production of trehalose. In this process, starch is liquefied using an α-amylase enzyme and this raw material is then converted into trehalose using four other enzymes.

3. The two most important of these four enzymes are produced by a strain of *Arthrobacter ramosus*. One enzyme converts the terminal (reducing) maltosyl unit of maltooligosaccharides to a trehalose unit. The other enzyme hydrolyses the α-1,4 glycosidic bond adjacent to the trehalose unit thereby liberating trehalose. In order to increase the yield of the process, two other enzymes (isoamylase from *Pseudomonas amyloderamosa* and cyclodextrin glucosyltransferase (CGTase) from *Bacillus stearothermophilus*) are used as ancillary enzymes. None of the source micro-organisms of these enzymes is genetically modified.

4. The application was prepared according to the European Commission's guidelines. Trehalose was identified as belonging to class 6 – foods produced using a novel process. The Committee consideration of the data provided is presented according to these requirements.

I. Specification of the Novel Food

Information on this aspect is provided in Chapter 2 of the application dossier. Supplementary information on a number of the aspects of the application was requested by the Committee during their deliberations. The supplementary information, in PDF format, has been placed on the website alongside this Initial Opinion.

5. Detailed specifications for trehalose are given in Chapter 2 of the application dossier. In addition, a specification for trehalose was agreed by the Food and Agriculture Organisation (FAO) when trehalose was considered by the Joint FAO/World Health Organisation Expert Committee on Food Additives (JECFA) earlier this year and to be published in FNP 52 edition 8 (2000). These specifications define that the product should be no less than 98% by dry weight, and are subsequently used as criteria for quality control testing of every batch. Proteinaceous impurities from the starch starting material and the different enzyme preparations used are removed by heat denaturation, followed by treatment with activated carbon and filtration. The activated carbon treatment also removes the majority of the other organic, non-ionic impurities. The ionic impurities are removed by a demineralisation step using cation and anion exchange resins. Any remaining inorganic salts are detected by the test for total ash, with a limit of 0.05%. Glycosidic impurities are removed during the crystallisation step and any excessive residues are detected by the HPLC analysis that forms the basis of the assay for trehalose. The Committee sought further detailed information concerning the operating conditions for the various purification steps to provide reassurance that no undesirable toxic substances would carry through into the trehalose end product. Analytical data in support of this specification were included in the confidential data in Annex 3 of the application dossier.

6. The supplementary information dossier included data from analysis of 5 batches of the stabilised enzyme preparation (Section 2(e)). The applicant has claimed confidentiality for these data and they are not included in the version intended for publication on the Internet, although the full data were considered by the Committee.

Discussion

Following consideration of the information provided by the applicant in the original submission, and the further information provided in the supplementary dossier, the Committee was content that the quality control mechanisms employed during the production process and the post-

production processing conditions applied were sufficiently robust to ensure that trehalose produced by the enzymatic process described in the dossier and complying with the JECFA (2000) specification to be published in FNP 52 edition 8 (2000), as agreed by FAO/WHO, is safe for use in food.

II. Effect of the Production Process Applied to the Novel Food

A detailed description of the process can be found in Section 3.3 (p 12-15) of the Company dossier. Critical control points for the process are detailed in Annex 4. The applicant has claimed commercial confidentiality for these data. Additional information on the safety of the MTSase/MTHase enzyme preparation was submitted by the applicant in the supplementary dossier.

Novelty of the Process

7. Trehalose extracted from yeast was the subject of an evaluation by the ACNFP in 1990. No indication was given by the applicant of whether the trehalose previously approved by the Committee was ever marketed in the Community. The subject of the present submission is a novel enzymatic process by which trehalose is produced from food-grade starch. Some of the enzymes used in this production process have not previously been used for food production in the Community. Trehalose produced by this enzymatic process is chemically identical to trehalose extracted from yeast.

General Description of the Process

8. In a first step, starch is liquefied by treatment with a thermophilic a-amylase, and then the obtained maltooligosaccharides are treated concurrently with maltooligosyl trehalose synthase (MTSase), maltooligosyl trehalose trehalohydrolase (MTHase), isoamylase, and cyclodextrin glucanotransferase (CGTase). Isoamylase is used as a debranching enzyme, cleaving α-1,6 glycosidic bonds of the starch molecule. CGTase is added in order to recycle maltose back into the process. Glucoamylase (from *Aspergillus niger*) and α-amylase (from *Bacillus subtilis*) are added to release any remaining trehalose moieties, and to degrade any remaining oligosaccharides and maltose to glucose. After completion of the trehalose- forming enzymatic step (saccharification), the reaction mixture is decolourised with activated carbon, filtered using diatomaceous earth and perlite as a filtering aid, de-ionised with ion exchange resins, and concentrated by evaporation. Trehalose is obtained by crystallisation.

Safety of raw material, chemicals and enzymes used in the process

9. Food grade starch is used as the starting material for the production process. The chemicals used as processing aids in the manufacturing process (calcium carbonate, calcium chloride, hydrochloric acid, sodium hydroxide, sodium carbonate, activated carbon, perlite, diatomaceous earth) have a purity suitable for use in the present process.

10. The thermophilic α-amylase (EC 3.2.1.1), which is used for starch liquefaction, is obtained from *Bacillus licheniformis*. The α-amylase from this source micro-organism has been examined by JECFA and been given an "ADI not specified". The MTSase (EC 5.4.99.15) and MTHase (EC 3.2.1.141) enzymes, which are crucial for the enzymatic synthesis of trehalose, are obtained from *Arthrobacter ramosus* (strain S34). The genus *Arthrobacter* is widely distributed in nature and is generally considered avirulent. Two batches of the MTSase/MTHase enzyme preparation from *Arthrobacter ramosus* were subjected to standard bacterial mutation tests and also to an acute toxicity test in rats. Detailed reports of these tests were presented to the Committee for consideration. The enzyme preparations were shown to produce no adverse effects in any of the tests.

11. The CGTase (EC 2.4.1.19) enzyme is obtained from a strain of *Bacillus stearothermophilus*. The safety of CGTase (from other source organisms) has been evaluated by JECFA in the context of the safety assessments of b- and g-cyclodextrin and has been given approval. The isoamylase (EC 3.2.1.68) enzyme from *Pseudomonas amyloderamosa* has been subjected to a range of toxicity studies, including mutagenicity studies and a 90-day toxicity study in the rat.

12. Glucoamylase (EC 3.2.1.3) enzyme from *Aspergillus niger* and a-amylase (EC3.2.1.1) from *Bacillus subtilis* are used in the last steps of the trehalose production process to degrade remaining oligosaccharides and maltose. The safety of these enzymes and source organisms has been evaluated by JECFA and specifications agreed.

Discussion

Detailed information was supplied describing the novel enzymatic process for producing trehalose, including the critical control points used to ensure that a consistent end product is obtained. The Committee noted that the MTSase and MTHase enzymes have not been evaluated for food use in Europe, and that they are produced by an organism that does not have a history of use in food products. However the Committee accepted that there is no statutory requirement in the EU for the approval of enzymes used as processing aids.

The Committee initially expressed concerns over the reproducibility of production of the enzyme preparations and considered that the possibility that the final trehalose product may contain unknown toxicants derived from the production organism had not been fully addressed. Further information on the proposed quality control and assurance programmes for the production of both the enzyme preparation and trehalose, and the post-fermentation purification steps, was subsequently supplied by the applicant. In addition, the applicant provided details of bacterial mutation tests and acute toxicity test in rats on the enzyme preparations, none of which showed any adverse reactions.

The Committee concluded that the supplementary information supplied by the company fully addressed the concerns that were raised during the initial deliberations on the application. The Committee was satisfied that the trehalose production process was fully controlled to produce a consistent trehalose end product and that the post-fermentation purification processes used would remove any unwanted impurities.

The Committee was satisfied that the detailed processing data provided regarding the enzymes used in the production of trehalose and the toxicological data supporting the safety of trehalose produced in this way, ensured that there were no safety concerns regarding the use of those enzymes preparations that had not been submitted for clearance in their own right for use in the production of trehalose, as described in the application dossier. However, for completeness and particularly if any other used for these enzymes were being considered, the Committee would strongly encourage the applicant to submit those enzyme preparations that have not yet been assessed to JECFA or a similar body for an evaluation of their general food safety.

IX. Anticipated intake/extent of use of the novel food

Information on this section of the application is provided in Chapter 5 of the application dossier, and in Annex 5 and 6.

Intended uses in food

13. Trehalose is 40-45% as sweet as sucrose and may be used to replace some of the sucrose in those types of food which require a certain amount of sucrose for technological reasons, but would have a more balanced taste profile if their sweetness was reduced.

14. Trehalose can be used to make fruit fillings and toppings, cream fillings, etc. which are microbiologically and physically as stable as those produced with sucrose but which have a richer flavour because trehalose is less sweet. In fruit preparations with a naturally high acid content there is less browning with trehalose than with sucrose because trehalose is more resistant to acid-catalysed hydrolysis.

15. Trehalose also acts as a stabiliser for proteins during freezing and drying. It has, for example, been found that enzymes retained a higher activity if they were dried in the presence of trehalose. It also stabilises phospholipid bilayers (such as liposomes) and more complex biological structures.

Current food applications of trehalose in Japan

16. Trehalose is used in Japan mainly in bakery products (cakes, frozen bread dough, cream fillings, toppings), beverages (sports drinks, fruit

drinks), hard and soft confectionery, fruit jam, breakfast cereals, rice, and noodles. It is used mainly to reduce sweetness (in bakery products and confectionery), to reduce moisture absorption (in breakfast cereals and certain types of confectionery), to reduce browning reactions (in beverages and certain types of confectionery) and to prevent starch retrogradation (in bakery products and noodles).

Estimated daily intake

17. The estimated daily intake (EDI) of trehalose from its different projected uses in food, but excluding chewing gum, has been calculated for the US population by ENVIRON (Arlington, VA) using the dietary survey approach. Though dietary habits of US and European consumers differ, an EDI calculation on the basis of US data was considered by the applicant to be adequate as the consumption of processed food is rather higher in the US than in the EU. Also, European food intake data are not available for conducting EDI calculations with a similar degree of accuracy.

18. This calculation model relies on food consumption data from the 1994-96 Continuing Survey of Food Intakes by Individuals in which data were collected from a representative sample of individuals residing in households in the US (approximately 15,000 individuals). Each individual was surveyed for two non-consecutive days using 24-hour recall interviews. The foods consumed were coded according to a system that contains about 6,000 different categories. For the purpose of the EDI calculation it was assumed that each food (or food component) that may contain trehalose, contained it at the highest feasible concentration. Where trehalose was used in a component of the food (such as in fruit-fillings), the intake of that component was calculated from data on food composition. The EDI of trehalose was calculated for each food category in which it could be used, and for all these food categories combined. Mean and 90th percentile intakes were calculated on per-user basis for children (2-12 years), teenagers (13-19 years) and adults (20+ years).

19. “Users” were defined as individuals who consumed food in the particular category on at least one occasion. Since food intake was recorded by time of day and by eating occasion [breakfast, brunch, lunch, dinner, supper, snack, and other (extended) eating occasion], the intake of trehalose could be calculated per eating occasion. For adults, the estimated exposure to trehalose is 5.6 and 13.0 g/day at the mean and 90th percentile, respectively. Mean intake by eating occasion (excluding extended eating occasions) ranged from 3.9 to 8.1 g/occasion, while intake at the 90th percentile ranged from 7.6 to 18.6 g/occasion. The highest estimated exposure to trehalose results from the intake of ice cream. In teenagers, this product results in an average intake of 16.7 g/day (intake may occur at more than one eating occasion).

20. The Applicant suggested that in assessing the total daily intake of trehalose from all dietary sources it is important to note that, with regard to gastrointestinal tolerance, the intake per eating occasion is a more important parameter than the combined total daily intake from all dietary sources. Considering the different anticipated uses of trehalose, the intake of trehalose is not concentrated at certain eating occasions (such as main meals) but is spread evenly over the day. This is also reflected by the data on estimated daily intakes. The data show that the mean and 90th percentile intake per eating occasion do not exceed 8.1 and 18.6 g, respectively, in any age group. A comparison between the intakes from the various food categories and the total intake from all sources demonstrates that many uses are mutually exclusive. Trehalose intakes per eating occasion are similar to the intake from one specific food category in a given age group.

Discussion

Estimated intakes per eating occasion are far below the 50-g intake, which is typically used in trehalose loading tests, and which is generally well tolerated. The intakes per eating occasion are also below the threshold dose for abdominal effects in particularly sensitive subjects [>30 g per eating occasion]. Adverse gastrointestinal side effects from the intended uses of trehalose, therefore, are not expected. Since in some applications trehalose may substitute for polyols, the total intake of low-digestible carbohydrates could even slightly decrease.

X. Information from previous human exposure to the NF or its source

Information on this aspect of the application is provided in Chapters 6 and 9 of the application dossier.

21. Trehalose occurs in bacteria, yeast (such as *S. cerevisiae*), a wide variety of fungi, algae, and a few higher plants. Intracellular trehalose appears to play an important role in the protection of the cells from dehydration and freezing, as well as from other adverse environmental conditions (heat shock, toxic levels of ethanol, osmotic stress). In addition, trehalose may serve as reserve carbohydrate during periods of carbon starvation.

22. Because of its presence in baker's and brewer's yeast, in which it reaches concentrations of up to 23% on a dry weight basis, small amounts of trehalose have been found in bread (1.2-1.5 g/kg dry weight), beer (45-240 mg/l), wine (44-129 mg/l), and honey (0.1-2.3 g/100g). Mushrooms, including many edible species, contain trehalose at levels of about 2-12 g/100g dry weight, but contents of up to 22% have also been reported. Trehalose produced by the enzymatic process described in this dossier became available in Japan for food use in 1995. By 1999, annual sales had reached 16-20,000 tons.

Discussion

The Committee agreed that trehalose itself is not a novel product and would have been consumed as a component of a variety of other foodstuffs.

XI. Nutritional information on the novel food

Information on this section of the application is presented in Chapters 7 and 9 of the applicant's dossier.

23. Ingested trehalose is digested by trehalase in the small intestine to glucose that is readily absorbed. The applicant considers that trehalose has the same physiological energy value and is nutritionally equivalent to glucose or maltose. Implications for individuals with trehalase deficiency are discussed in paragraphs 58-9.

Discussion

The committee was content with the information provided by the applicant.

XII. Microbiological information on the novel food

Information on this aspect of the application is provided in Chapter 8 of the application dossier

24. The enzymes that are used in the novel production process of trehalose are obtained from non-genetically modified strains of *Arthrobacter ramosus, Pseudomonas amyloderamosa, Bacillus stearothermophilus, and Bacillus licheniformis*. In addition to filtration of the enzyme-containing fermentation broths, the trehalose production process comprises several heat-treatment steps. Therefore, the inadvertent presence of micro-organisms in the final product is unlikely and, should it occur, it would be detected by the proposed quality control procedures and dealt with accordingly.

Discussion

The Committee was content with the information provided by the applicant and considered that the quality control procedures described would be adequate to detect any inadvertent microbiological contamination of the trehalose product.

XIII. Toxicological assessment of the novel food

Information on this section of the application is presented in chapter 9 of the company's dossier and Section 1 of the supplementary information dossier.

25. The material used in toxicological studies was 99% pure trehalose, whereas the commercial specification defines material of ≥98% purity. This apparent discrepancy in purity was clarified by the applicant. The former value is a measured amount for the batch(es) used in the studies whilst the value for the general specification is a minimum quality that should be attained from the production process. When trehalose of 99.1% purity was analysed by HPLC, the main impurity was glucose (0.5%), along with a-D-maltosyl- a-D-glucoside (0.3%) and a-D-isomaltosyl- a-D-glucoside (0.1%) (Section 3.5.2 of the original application dossier). This information was reiterated in the additional information supplied by the applicant. Following further consideration the Committee was content that the quality assurance parameters employed during the production process, along the subsequent post-production processing steps used, would ensure that the final trehalose product contains no unknown toxicants.

Subchronic oral toxicity study in mice

26. The company has carried out a subchronic oral toxicity study in mice to OECD (No: 408) guidelines in compliance with GLP. Four groups of 20 male and 20 female NMRI mice per group were fed trehalose in the diet at concentrations of 0 (control), 0.5, 1.5, or 5% for 13 weeks. These dietary concentrations correspond to mean intakes (for both sexes) of 0, 840, 2500, and 8300 mg/kg body weight per day.

27. During the study four males died or were killed in *extremis*, No: 78 (high dose group), 57 (mid dose group) 12 (control) and 16 (control). Animals No. 78 and 12 exhibited a severe reduction in food intake prior to death, the other two males died from other non-treatment related causes.

28. Throughout the treatment period food consumption in males was reduced in the high dose group, often attaining statistical significance, and to a lesser extent in the mid dose group. However, because this reduction in food consumption was apparent from the start of the study it is attributed to the unpalatability of high concentrations of trehalose. Food consumption in females was unaffected. Body weight gain in males was only slightly retarded in the top two dose groups; body weight gain in females was unaffected.

29. Clinical signs (evaluated daily) and opthalmoscopic evaluation (at start and end of study in control and high dose group) were unaffected by treatment. Blood and urine were sampled at weeks 5, 9 and 13. Haematology parameters were unaffected by treatment. While there were a few significant intergroup differences, these were not considered treatment-related as there was no dose-response. Urinalysis did not reveal any treatment-related effects.

30. There was a significant decrease in plasma bilirubin in high dose males at weeks 5 and 9 and in high dose females at week 5. However, the magnitude of the reduction was slight, within the limits of historical controls, and therefore not considered treatment related. Plasma glucose was elevated in the high dose group in both males and females at all three sampling times, significantly so at weeks 5 and 9 in females. Glucose concentrations were also raised on occasions in the low and mid dose groups. These increases are attributed to the metabolism of trehalose to glucose. Plasma calcium concentrations were significantly reduced in the top two male groups at week 13; however, concentrations were unaffected in weeks 5 and 9 in males and at all time points in females and so these increases are not considered treatment related. Plasma potassium concentrations were only determined at week 13 for which there was a dose-related decrease in both sexes, significantly at mid and high dose groups in males and high dose group in females. While the potassium concentrations in controls were slightly higher than expected, an association with treatment cannot be excluded, though the changes were all within the limits of historical controls. In contrast, in females there was a small dose-related increase in plasma phosphorus concentrations, which was significant at weeks 5 and 9 in the top dose group. This trend was also slightly evident in males at week 13 only and though phosphorus concentrations were also significantly increased in the high dose group at week 5 they were lower than controls on week 9 and thus these findings are not considered treatment related. While there were no treatment-related intergroup differences in aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) activity, extremely high AST, ALT, and LDH activity was measured in one high dose male (No: 72) at week 5. However, these high values were not evident at weeks 9 and 13 in this animal or in any other animal at all times and are therefore not considered treatment related.

31. Adrenals, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, testes, thyroid and thymus weights were recorded at necropsy and data for absolute weight and organ:body weight and organ:brain weight ratios presented, none of which were significantly affected by treatment.

32. At necropsy there were no treatment-related gross pathological changes. Representative tissue samples from an extensive number of organs from animals from groups 1 (control) and 4 (high dose) were subject to histological evaluation as were any tissues from groups 2 and 3 exhibiting gross pathological change at necropsy or from any unscheduled deaths (No: 57). There were no treatment-related histological findings. Animal 78, killed *in extremis*, had severe pyelonephritis but this was not considered treatment-related; at necropsy, gravel (a term used by the authors to presumably refer to salt deposits) was detected in the urinary

bladder and right kidney. Animal 12 had extreme thoracic inflammation, which the authors conclude contributed to the animal's death.

33. In conclusion, the administration of trehalose to mice for 13 weeks at dietary concentrations of up to 5%, equivalent to 8300 mg/kg body weight per day, was well tolerated with no evidence of toxicity. Thus, the No Observed Adverse Effect Level (NOAEL) can be considered to be 8300 mg/kg body weight per day.

Oral two generation reproduction study in rats

34. The company has carried out an oral two generation, one litter per generation, reproduction study in rats, to OECD (No: 416) guidelines in compliance with GLP.

35. Four groups of 28 male and 28 female albino rats per group (F_0 generation) were fed trehalose in the diet at concentrations of 0 (control), 2.5, 5, or 10% for 10 weeks prior to mating, throughout mating and gestation periods and during lactation until they were killed. After weaning, the F_0 male and female parents were killed and subjected to necropsy. The total litter size, sex ratio, number of stillbirths and livebirths, live and dead pups, pup weight, and external abnormalities in the F_1 generation, were recorded on postnatal day (PND) 1, 4, 7, 14, and 21 where appropriate. On PND 4, litters were standardised to 4 males and 4 females per litter. All stillborn pups, pups found dead, or pups killed *in extremis* were subject to necropsy (as were similar pups of the next (F_2) generation). After weaning, 28 male and 28 females from the F_1 generation were randomly selected to rear the F_2 generation (mating of siblings was avoided; animals mated were over 10 weeks old). The non-selected F_1 animals were discarded. The selected F_1 animals were administered trehalose at the same dietary concentrations as the F_0 generation until weaning of the F_2 generation, after which the F_1 parents were killed and subjected to necropsy. The procedures followed to rear the F_2 generation litter were reported to be identical to those used to rear the F_1 generation litter; litter size was standardised on PND 4 and the same set of observations/measurements taken. The following organs from control and high dose F_0 and F_1 animals were subject to histological investigation plus all organs exhibiting gross pathological change at necropsy: ovaries, uterus, vagina, testes, epididymides, seminal vesicles (with coagulating glands and their fluids), prostate, pituitary, and spleen. In addition, the reproductive organs of males that failed to sire and non-pregnant females of the low and mid dose groups were histologically examined. At necropsy the spleen was weighed (there is no justification as to why only the spleen was weighed).

36. Clinical signs (checked at least once daily) during premating, gestation, and through to weaning were unaffected by treatment. While there were a few significant changes in body weight and body weight gain

in both the F_0 and F_1 generations, these changes were not consistent and exhibited no dose-response and were therefore not considered to be treatment-related. While food consumption was significantly increased (though decreased on one occasion) on a number of occasions in both generations, primarily during the pre-mating periods, these changes were not consistent and were therefore not considered to be treatment-related. The mean intake of trehalose for F_0 and F_1 males was 1.7, 3.5, and 7.1 g/kg body weight per day in the low, mid and high dose groups respectively. For F_0 and F_1 females, the corresponding intakes during the premating period were 1.9, 3.7, and 7.1 g/kg b.w./day. During the gestation period, the intakes were 1.5, 3.1 and 6.2 g/kg b.w./day and the lactation period were 3.3, 6.9 and 14 g/kg b.w./day.

37. At necropsy of both parental generations, absolute and relative spleen weight were unaffected by treatment. There were very few gross pathological changes, none of which were considered to be treatment-related. There were a number of histological changes, though they occurred with low incidence and without a dose-response and therefore were not considered to be treatment-related.

38. In both generations there were no treatment-related effects on the fertility and reproductive parameters assessed, namely: precoital time, mating index, male and female fertility, female fecundity index, gestation index, duration of gestation, and post-implantation loss (though no details are provided on how the number of implantation sites were assessed).

39. Trehalose had no consistent adverse effects on litter size, the number of liveborn pups (the number of liveborn pups was significantly increased in the high dose group of the F_0 generation and mid and high dose groups of the F_1 generation), the number of stillborn pups (the number of stillborn pups was significantly decreased in the high dose group of the F_0 generation), sex ratio, pre-cull (days 1-4) pup mortality (pup mortality was significantly decreased in the low and high dose F_0 groups), post-cull pup mortality, and sex ratio. It was reported that no grossly malformed pups were observed and the results of necropsy of stillborn pups, pups that died or were killed *in extremis* did not indicate any abnormal development. There were no treatment-related effects on mean pup body weight and pup body weight changes. Clinical signs in the pups from PND1 to weaning were unaffected by treatment when evaluated on a litter basis in both generations. On an individual pup basis there were a number of significant inter-group differences; however, none of these clinical signs exhibited a dose-response or were consistent across generations and therefore were not considered to be treatment-related.

40. In conclusion, dietary administration of trehalose at dietary concentrations up to 10% had no effects on reproduction of the parental F_0 and F_1 generation or the development of F_0 and F_1 generation pups. Taking

the lowest recorded intake of the high dose group, the NOAEL can be considered to be approximately 6g trehalose/kg body weight per day.

Developmental study in rabbits

41. The company has carried out a developmental study in New Zealand white albino rabbits to OECD (No: 414) guidelines in compliance with GLP.

42. Four groups of 16 artificially inseminated female rabbits were administered trehalose in the diet at concentrations of 0 (control), 2.5% (low), 5% (mid) and 10% (high) from gestational day (GD) 0 (day of a.i.) to GD 29. These dietary concentrations correspond to intakes of 0, 540, 1100, and 2000 mg trehalose/kg body weight per day throughout gestation. On day 29 the animals were killed and subject to necropsy. Reproductive organs were weighed and examined. Foetuses from all dose groups were examined for external and visceral abnormalities and, with the exception of foetuses from the low dose group, were also examined for skeletal abnormalities.

43. During the study one high dose animal (No: 113) was killed *in extremis* as it was not eating and another high dose animal (No: 117) was found dead. At necropsy both animals were found to have a hairball in their stomachs. Though the cause of death of No: 117 was unknown, neither the death of No: 117 nor the moribund condition of No: 113 were considered treatment related.

44. Clinical signs in all animals were unaffected by treatment. In those animals that were pregnant there were no significant differences in mean body weights, body weight changes and food consumption. At necropsy, there were no treatment-related gross pathological changes.

45. Twelve, twelve, fourteen, and thirteen animals (including the two dead animals and one animal that underwent an unscheduled early delivery) from the control, low, mid, and high dose groups respectively were found to be pregnant. In those animals pregnant at GD29, the number of corpora lutea, implantations, early/late resorptions, live and dead foetuses, and sex ratio of the foetuses were unaffected by treatment. Carcass and ovaries weight and net weight change (body weight gain during gestation minus gravid uterine weight) were unaffected by treatment. However, in the high dose group, the gravid uterus weight was higher than controls and this difference was significant when empty uterus weight (i.e. uterus weight minus foetuses and placenta) was compared. The authors attribute this increase to the (non-significant) higher number of foetuses in the high as opposed to the control group (8.4 verses 6.5 respectively). The mean foetal weights and placental weights did not differ significantly between treatment and control groups. Aside from one control foetus with proboscis and ectopic eyes, no external foetal abnormalities were observed. There were

no treatment-related placental and visceral foetal abnormalities. With regards to skeletal abnormalities, there was a significant increase in the incidence of accessory ribs in the mid-dose, though not the high-dose, group when expressed on a foetal basis, though not when data for litters were analysed and for these reasons this effect was not considered to be treatment-related. The incidence of unossified distal epiphysis of humerus was significantly increased in the mid-dose group when expressed on a foetal basis, though not when data based on litters were analysed. However, the incidence in the high dose group was not significantly different from controls and thus this effect was not considered to be treatment-related. The incidence of one or two incomplete ossified thoracal bodies was significantly increased, when expressed on a foetal basis, in the mid and high dose groups, though when expressed on a litter basis this was only significant in the mid dose group. However, the incidence of three or more incompletely ossified thoracal bodies was slightly decreased in the mid and high dose groups. Furthermore, the incidence of total incompletely ossified thoracal bodies was only significantly increased when expressed on a foetal basis in the mid-dose group and thus these effects were not considered to be treatment-related.

46. In conclusion, trehalose did not induce maternal or developmental toxicity at concentrations up to 10% in the diet, equivalent to a dietary intake of 2 g trehalose/kg body weight per day throughout gestation.

Developmental study in rats

47. The company has carried out a developmental study in Wistar rats to OECD (No: 414) guidelines in compliance with GLP.

48. Four groups of 28 pregnant rats were administered trehalose in the diet at concentrations of 0 (control), 2.5% (low), 5% (mid) and 10% (high) from gestational day (GD) 0 to GD 21. These dietary concentrations correspond to intakes of 0, 1.7, 3.5, and 6.9 g Trehalose/kg body weight per day throughout gestation. On day 21 the animals were killed and subject to necropsy. Reproductive organs were weighed and examined. Foetuses from all dose groups were examined for external abnormalities and foetuses from the control and high dose groups were also examined for visceral and skeletal abnormalities.

49. There were no unscheduled deaths during the study. Aside from one high dose and one control animal exhibiting haemorrhagic discharge there were no other remarkable clinical observations. At necropsy there were no treatment-related gross pathological changes. In those animals that were pregnant there were no significant differences in mean body weights, body weight changes and food consumption.

50. During the study 22 (including 1 early delivery), 25 (including 2 early deliveries), 24, and 24 females in the control, low, mid, and high dose groups were pregnant respectively. In those animals that were pregnant there were no significant differences in mean body weights, body weight changes and food consumption. In those animals pregnant at GD21, the number of corpora lutea, implantations, early/late resorptions, live and death foetuses, and sex ratio of foetuses were unaffected by treatment. In these animals there were no significant differences in empty and gravid uterus weight, ovaries weight, carcass, and net weight change. The mean foetal weights and placenta weights did not differ significantly between treatments and controls. The incidence of large foetuses (that is foetal weight >125% of mean foetal body weight) was significantly reduced in the low and high dose groups (though was unaffected in the mid dose group). However, the incidence of small foetuses (that is foetal weight <75% of mean foetal body weight) was also significantly reduced in the low dose group and, though not significantly, also in the high dose group. For this reason and the lack of a dose-response, these effects on foetal size were not considered to be treatment-related. There were no treatment-related external foetal or placental abnormalities. In foetuses from the control and high dose groups that were pregnant at GD21, there were no treatment-related visceral or skeletal abnormalities.

51. In conclusion, trehalose did not induce maternal or developmental toxicity at concentrations of up to 10% in the diet, equivalent to a dietary intake of 6.9 g Trehalose/kg body weight per day throughout gestation.

Genotoxicity data on trehalose

52. Three mutagenicity assays have been submitted by the company, two *in vitro* and one *in vivo* assay, all in compliance with GLP.

Gene mutation in bacteria

53. This study was carried out to OECD guideline Nos: 471 and 472. Four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and a tryptophan-dependent mutant of *Escherrichia coli* were exposed to trehalose (purity: 100%) at test concentrations of 312.5, 625, 1250, 2500 and 5000 µg/plate in two independent experiments. The test concentrations were determined from a dose range finding cytotoxicity study, in the presence and absence of S9 mix of Aroclor 1254 pre-treated rats. Distilled water was used as solvent and negative control. N-Ethyl-N'-nitrosoguanidine and 2-aminoanthracene were used as positive control in the absence and presence of an activation system respectively.

54. No cytotoxicity was observed with any of the test concentrations. The test substance did not induce gene-mutations in any of the tester strains. The positive controls produced the expected increase in mutant

frequencies. Therefore, the test substance was not regarded to be mutagenic under the conditions of this test.

Chromosomal aberrations in cultured mammalian cells

55. Trehalose (purity: 99.2%), was tested in the chromosomal aberration assay using Chinese Hamster Ovary cells in the presence and absence of S9 mix in two independent experiments. Sterile water was used as the solvent. In experiment 1 cells were exposed to trehalose for 3 hours in the presence and absence of an activation system. On the basis of the cytotoxicity assay, the concentrations of trehalose tested in the first assay were 1250, 2500 and 5000 µg/ml; metaphase cells were then harvested at 21hr after initiation of exposure. The replicate assay employed the same concentrations of the test substance and cultures were exposed to trehalose for 21hr in the absence of an activation system and for 3hr in its presence. Cultures in the replicate experiment were then harvested at 21hr and in the case of the control and high dose, at 45 hr. The 45-hr cultures were also examined for polyploidy at the 5000 µg/ml dose. Methyl methanesulfonate and cyclophosphamide were used as positive controls in the absence and presence of an activation system respectively.

56. No cytotoxicity was observed in the presence or absence of an activation system when Trehalose was tested up to 5000 µg/ml. Trehalose did not produce chromosomal aberrations or induce polyploidy in either experiment. Therefore, the test substance was not regarded to be clastogenic under the conditions of this test.

Micronucleus assay

57. Trehalose (purity 99.2%) was tested in the mouse bone marrow micronucleus test using male and female mice. The compound was administered by a single intraperitoneal dose to groups of 10 animals per sex. Five mice per sex were sacrificed at 24 or 48 hours post treatment for the assessment of cytotoxicity and micronucleus formation. The compound was dissolved in sterile water and tested at the following doses: 1250, 2500 and 5000 mg/kg body weight. A vehicle control group and a cyclophosphamide positive control group were also evaluated. Two principal parameters were determined using 1 slide/animal 1) the number of polychromatic erythrocytes (PCE) among 200 total erythrocytes (RBC) per animal and 2) the number of micronucleated RNA positive erythrocytes (MPE) per 2000 PCE per animal.

58. There were no clinical signs or early deaths reported. Trehalose produced a negative response in this assay. The positive control produced the expected increase in micronucleated cells. Therefore, the test substance was not regarded as being clastogenic under the conditions of this test.

Human studies

59. Trehalose is rapidly metabolised in the gut to glucose by the brush border enzyme trehalase. A small minority of the population exhibits a primary (hereditary) or secondary (acquired) trehalase deficiency and thus may experience intestinal discomfort such as laxation, after ingestion of excessive amounts of trehalose due to the osmotic activity of undigested trehalose in the gut. However, smaller amounts of trehalose are tolerated by such individuals without any such symptoms. The prevalence of trehalase deficiency is low; the company suggests, on the basis of a limited number of studies, that the prevalence in western populations is rare, much less than that for lactase deficiency (Murray et al British Journal of Nutrition (2000) Vol 83(3) p 241-245).

60. The company has summarised a number of studies investigating intestinal tolerance following single bolus oral doses of trehalose in healthy participants. More than 100 participants are reported to have ingested trehalose at single doses up to 20-30 g without the occurrence of gastrointestinal symptoms. At higher doses gastrointestinal symptoms such as flatulence, watery stool, and distension were reported to occur. In trehalase-deficient individuals such effects are likely to occur at lower trehalose intakes. However, the intake of trehalose that would be tolerated by such individuals is unclear though the severity of any such gastrointestinal effects will also be dose-dependent. Only one study has investigated trehalose tolerance in individuals with self-reported mushroom intolerance as a surrogate of trehalose intolerance. Mushrooms area natural source of trehalose and individuals with trehalase deficiency may only recognise that they are mushroom-intolerant. While the interpretation of this study is limited by its design, mushroom intolerance was not limited to those individuals with trehalase deficiency as determined by trehalase activity of gut biopsies. Furthermore, the rise in plasma glucose concentrations after trehalose intake did not differ between subjects with and without reported gastrointestinal symptoms.

Discussion

A number of animal toxicological studies have been conducted on trehalose produced by this enzymatic process. The administration of trehalose to mice for 13 weeks at dietary concentrations of up to 5%, equivalent to approximately 8g trehalose/kg body weight per day, was well tolerated with no evidence of toxicity. Furthermore, trehalose did not cause maternal nor developmental toxicity in rabbits and rats when administered at dietary concentrations up to 10%, equivalent to approximately 2 and 7 g trehalose/kg body weight per day respectively. In a two-generation reproduction study in rats trehalose had no effects on reproduction and development when administered at dietary concentrations up to 10%, equivalent to 6 g trehalose/kg body weight per day.

Trehalose is a naturally occurring disaccharide (carbohydrate), that consists of two glucose molecules linked by a glycosidic bond. It is metabolised in the gut and is absorbed as glucose. The potential subchronic, reproductive and developmental toxicity of trehalose has been investigated in a number of studies in animals and was well tolerated at dietary concentrations up to 10%. Furthermore, trehalose was not shown to be mutagenic. While no chronic/carcinogenicity studies with trehalose have been conducted, these are not considered necessary in assessing the safety of trehalose in light of the available toxicological data and the fact that trehalose is completely metabolised to glucose.

Predicted dietary intakes of trehalose by the mean and high-level (90th percentile) consumer are estimated as 8 and 19 grams per day respectively. These intakes are lower than the doses of trehalose that were ingested by healthy participants (up to 30g) without the occurrence of gastrointestinal symptoms and thus adverse gastrointestinal effects in the general population from the intended uses of trehalose are not expected. In individuals who are deficient in the enzyme trehalase that breaks down trehalose, and are thus trehalose intolerant, gastrointestinal symptoms may occur at lower intakes. However, trehalose intolerance is estimated to affect <1% of the population.

The Committee noted that some individuals who believe themselves to be intolerant to mushrooms may, in fact, be deficient in the enzyme trehalase. Such individuals would also be intolerant of foods containing significant amounts of trehalose produced by this enzymatic process, although they would be able to tolerate foods containing small amounts of trehalose. However not all individuals with mushroom intolerance are deficient in the enzyme trehalase.

The Committee considered that, given the wide range of toxicological information supplied by the applicant, trehalose produced by this enzymatic process is safe for use within the range of foodstuffs detailed by the company.

OVERALL DISCUSSION

61. The application contains good specification data and a detailed description of the production process. The process is well controlled and consistent product is produced. A specification for trehalose produced by this enzymatic process has been agreed by FAO.

62. There are no nutritional concerns for the product as trehalose is readily converted to glucose. The eating occasion data provided show that there was no glucose overload on the occasions when the trehalose was consumed.

63. The trehalose produced by this enzymatic process has been shown to be non-toxic and non-mutagenic. Many of the proposed uses for trehalose are mutually exclusive and there are sufficient safety factors between the predicted intake of the product and the level tested in experiments. There was a 60x safety margin between the proposed average intake of trehalose and the highest dose tested in animals and a 20x safety margin between the proposed extreme (90th percentile) intake and the highest dose tested.

64. No information was provided in the application dossier concerning proposals for labelling of the product. During their deliberations, members of the ACNFP were concerned that diabetics may be unaware that trehalose is a disaccharide of glucose, and not take it into consideration when managing their dietary calorific intake

65. It was therefore suggested that it might be helpful to include the description ‘a sugar’ after the name trehalose in the ingredient list, and the Committee therefore sought the advice of the UK Food Advisory Committee on the labelling requirements for trehalose in relation to the needs of diabetics. Taking account of the advice received, the Committee recommends that trehalose should be listed as an ingredient in the foods to which it is added. In addition, trehalose content should be taken into account when determining nutritional labelling information, particularly the content of sugars and carbohydrates in food products, so that diabetics are fully able to manage their overall calorie intake. The Committee was advised that there are no general powers to add a description such as ‘a sugar’ to the name trehalose in the ingredient list. In addition, under the EC Food Labelling Regulations, the term ‘sugar’ is a reserved generic description that may only be used for ingredients that are ‘any type of sucrose’ and may therefore not be used as a description to accompany trehalose. Furthermore, other materials, such as maltose and lactose, that may also be added to a range of food products, are not described in this way, and thus there is no precedent for such additional information on the labels of foods containing trehalose. Nevertheless, the Committee considers that information should be provided to health professionals caring for diabetics and to the relevant support groups, so that diabetics are aware that trehalose is a source of glucose. This approach has been adopted in the past to ensure that diabetics have an above average knowledge of the nutritional quality of food.

66. The Committee noted that some of the enzymes preparations used to produce trehalose have not been formally assessed for safety in their own right. However, the Committee was satisfied that the detailed processing information provided, together with the range of toxicological data on trehalose produced using this process, provided sufficient reassurance as to their safety for this particular use. However, the

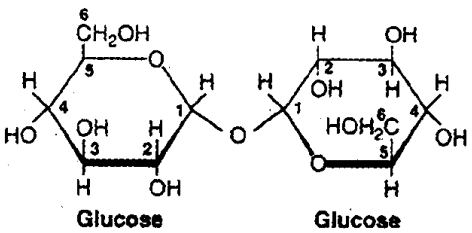
Committee agreed that the applicant should be strongly encouraged to submit as soon as possible, for formal evaluation, information on the enzyme preparations used in the trehalose production process that have not yet been evaluated for their general food safety, particularly if other food uses are anticipated/.

CONCLUSION

67. The Advisory Committee on Novel Foods and Processes is satisfied that trehalose produced by the novel enzymatic process described and complying with the specification agreed at the 55th JECFA and to be published in FNP 52 edition 8 (2000) can be approved as a novel food ingredient, to be used in the range of foodstuffs detailed in the application dossier.

Trehalose – Specification (slightly revised specification was agreed at the 55th JECFA (2000) and will be published in FNP 52 edition 8 (2000)

SYNONYMS	α, α – trehalose
DEFINITION:	A non-reducing disaccharide that consists of two glucose moieties linked by an a, 1,1-glucosidic bond. It is obtained from liquefied starch by a multi-step enzymatic process. The commercial product is the dihydrate.
Chemical name	α -D-glucopyranosyl-a-D-glucopyranoside
C.A.S. number	6138-23-4 (dihydrate)
Chemical formula	$C_{12}H_{22}O_{11} \cdot 2H_2O$ (dihydrate)
Structural formula	



Formula weight	378.33 (dihydrate)
Assay	Not less than 98% on an anhydrous basis.

DESCRIPTION CHARACTERISTICS	Virtually odorless, white or almost white crystals with a sweet taste.
IDENTIFICATION	
Solubility	Freely soluble in water, very slightly soluble in ethanol.
Specific rotation	$[\alpha]_D^{20}$ +199° (5% aqueous solution).
Melting point	97°C (dihydrate)
PURITY	
Loss on drying	Not more than 1.5% (60°C, 5 h) (Crystal water of dihydrate is not released under these conditions).
Total ash	Not more than 0.05%.
Lead	Not more than 1mg/kg. Prepare a sample solution as directed for organic compounds in the Limit Test and determine by atomic absorption spectroscopy, appropriate to the specified level.
Microbiological criteria	Total (aerobic) plate counts: < 300/g *Coliforms*: Negative by test Salmonella: Negative by test Yeast and molds <100/g.

METHOD OF ASSAY

Principle: Trehalose is identified by liquid chromatography and quantified by comparison to a reference standard containing standard trehalose.

Preparation of sample solution: Weigh accuratelyabout 3 g of dry sample into a 100-ml volumetric flask and add about 80 ml of purified, deionized water. Bring sample to complete dissolution and dilute to mark with purified deionized water. Filter through a 0.45 micron filter.

Preparation of standard solution: Dissolve accurately weighed quantities of dry standard reference trehalose in water to obtain a solution having known concentration of about 30 mg of trehalose per ml.

Apparatus: Liquid chromatograph equipped with a refractive index detector and an integrating recorder.

Conditions:

Column:	Shodex Ionpack KS-801 (Showa Denko Co.)
-length:	300 mm
-diameter:	10 mm
-packing:	Shodex Ionpack KS-801
-temperature:	50°C

Solvent: Water

Flow rate: 0.4 ml/min

Injection volume: 8 μl

Procedure: Inject separately equal volumes of the sample solution and the standard solution into the chromatograph. Record the chromatograms and measure the size of response of the trehalose peak.

Calculate the quantity, in.mg, of trehalose in 1 ml of the sample solution by the following formula:

$\%\ \text{trehalose} = 100 \times (R_U/R_S)\ (W_S/W_U)$

where

R_S = peak area of trehalose in the standard preparation

R_U = peak area of trehalose in the sample preparation

W_S = weight in mg of trehalose in the standard preparation

W_U= weight of dry sample in mg.

APPENDIX III

Patrick Deboyser
European Commission DG Sanco
Rue de la Loi 200
B-1049 Brussels
Belgium

Reference: NFU 19

17 July 2000

Dear Mr Deboyser

Application for Authorisation to Market Fruit Preparations Pasteurised Using a High Pressure Treatment Process

At its forty sixth meeting on 6 July, the Advisory Committee on Novel Foods and Processes (ACNFP), the UK Competent Assessment Body, considered the French Competent Authority's Initial Opinion on the above application from Danone.

The ACNFP generally agreed with the opinion of the French Competent Authority and accordingly the UK Competent Authority is content for clearance to be given **for the fruits listed when processed in the manner described in the application dossier only**, subject to the following conditions:

Significant changes to the operating conditions or to the types of foods to be processed would require a further application for approval.

The ACNFP was concerned that, as high pressure processing does not inactivate bacterial spores, products processed in this way could represent a risk to consumers of botulism poisoning. The ACNFP agreed that approval for the use of the high pressure treated fruit preparations should be limited only to final products whose characteristics conformed with the criteria recommended in the enclosed report published by the UK Advisory Committee on the Microbial Safety of Food (ACMSF) in 1992, and amended in 1995.

In particular, in addition to chill temperatures, which should be maintained throughout the chill chain, the following controlling factors should be used singularly or in combination to prevent growth and toxin production by psychrotrophic *Clostridium botulinum* in prepared chilled foods with an assigned shelf-life of more than 10 days;

- A heat treatment of 90°C for 10 minutes or equivalent lethality,
- a pH of 5 or less throughout the food and throughout all components of complex foods,
- a minimum salt level of 3.5% in the aqueous phase throughout the food and throughout all components of complex foods,
- an a_w of 0.97 or less throughout the food and throughout all components of complex foods.

Where chilled storage is the sole controlling factor, chilled foods stored between 5°C and 10°C should have an assigned shelf-life of 5 days or less. If a shelf life of up to 10 days is required, the chilled storage temperature should be 5°C or below.

The ACNFP agreed with the French CA Initial Opinion that high-pressure processing would not introduce into fruit products further allergens that were not already present in unprocessed fruit. However, the ACNFP noted that, as high pressure processing is a mild treatment that may not denature potential allergens, this could have implications for susceptible individuals who are allergic to unprocessed fruit, but not thermally processed fruit.

Yours sincerely

Sue Hattersley
ACNFP Secretariat

Cc: Competent Authorities, Ms A. Davi (Groupe Danone)

APPENDIX IV

Mr A Klepsch
DG Sanco
European Commission
Rue de la Loi 200
B-1049 Brussels
Belgium

Reference: NFU 164

10 August 2000

Dear Mr Klepsch

APPLICATION FOR AUTHORISATION TO MARKET BT 11 SWEET MAIZE (NOVARTIS) UNDER EC REGULATION 258/97

The UK Competent Authority has considered the above application and has identified a number of concerns regarding the information provided in the dossier and the safety assessment of this application.

The quality and scope of the molecular biology data has raises a number of important questions. The Southern blot data suggests there is an extra fragment of DNA that hybridises along with the main band (Panel A, Page 6, Appendix 3). It is possible that an additional fragment of DNA has become inserted at a different location from the main insertion site and the nature of this faint band therefore needs to be clarified. The quality of the blots in general is too poor to determine if other adventitious bands occur with other blots.

Digestibility and acute oral toxicity studies on the Cry 1A protein were carried out on protein expressed in *E. coli* rather than the protein expressed in the sweet maize. Other *in vivo* studies were based on studies in tomato. Although the recent FAO WHO report on safety of GM foods derived from plants recognises the merits of testing material derived from analogous systems it stresses the need to demonstrate such material is biochemically and functionally equivalent to that produced in the genetically modified food. The applicant should be asked to demonstrate such equivalence.

Studies relating to expression of the PAT protein were carried out in field maize rather than on the sweet maize. Although it is accepted that the protein is the same as in the field maize there is no indication of the expression levels of the pat protein in the sweet maize. The data collected

on sweet maize examines herbicide tolerance to indicate the presence of the *pat* gene rather than looking at the levels of PAT protein in the kernels, which will be eaten.

Based on the ACNFP's advice, processed products from Bt 11 field maize and hybrids derived from it were cleared for food use in February 1997. However, as it is likely that some of the Bt11 sweet maize will be eaten unprocessed, it is essential that before approval is given studies relating to the expression of the introduced genes are addressed and that data is provided on the sweet maize rather than from its parental field maize.

Yours sincerely

Dr Clair Baynton

Cc Dr Patricia Ahl Goy, Novartis
All Member States

APPENDIX V

Mr A Klepsch
European Commission
DG Sanco
Rue de la Loi 200
B-1049
Brussels

Reference: NFB295

26 April 2000

Dear Mr Klepsch

APPLICATION UNDER EC REGULATION 258/97 FOR FOOD AND FOOD INGREDIENTS DERIVED FROM ROUNDUP READY MAIZE LINE GA21

Further to my letter of 14 April I wish to inform you that the UK Competent Authority, having considered the data provided by the applicant in response to our earlier request, is seeking clarification on the following aspects of the application.

The applicant argues that the modified EPSPS gene has no significant sequence homology to known allergens and toxins, citing data in a Monsanto Technical Report (MSL 15168), please could the company supply a copy of this report. There are now accessible international programs (for example PROPSEARCH) which clearly indicate that it is possible to find remote homologies within the same fold and most often similar function with insignificant alignment homology with respect to amino acid sequence. Sequence similarities below a defined threshold might still be functional or share structural homology which has particular importance with respect to allergenicity. The applicant should be asked to confirm the percentage cut off point they used when considering significant homology to known allergens and toxins.

The company goes on to suggest that gastric and post gastric digestibility studies are sufficient to discount GI allergenicity. This evidence is contained within a Monsanto Technical Report (MSL 15169), please could the company supply a copy of this report. A molecule such as mEPSPS may exert sensitising effects from the oral mucosa as much as from the lower GI tract. Has the company performed any experimental work in animal systems to assess this or addressed this issue in any other way?

The application requested approval of the unprocessed grain and products derived from it. Since there is no assessment on the environmental impact of the GA21 maize in this application, the approval should be restricted to processed foods and food ingredients derived from this line. We note however that an application is still pending under EC Directive 90/220.

Yours sincerely

Nick Tomlinson

Cc Dr TGA Clemence, Monsanto

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